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AWARD NUMBER DAMD17-96-C-6093

TITLE: Hormonal Contraception, Body Water Balance and Thermoregulation

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REPORT DATE: October 1998

TYPE OF REPORT: Annual

PREPARED FOR: Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 1998	Annual (15 Sep 97 - 14 Sep 98)

4. TITLE AND SUBTITLE
Annual Report: Hormonal Contraception, Body Water Balance and Thermoregulation

5. FUNDING NUMBERS
DAMD17-96-C-6093

6. AUTHOR(S)
Ethan R. Nadel, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
The John B. Pierce Laboratory
New Haven, Connecticut

8. PERFORMING ORGANIZATION REPORT NUMBER
01.130-02

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
504 Scott Street
Fort Detrick, Maryland 21702-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

19990614 048

13. ABSTRACT *(Maximum 200 words)*

To test the hypothesis that estrogen enhances water and sodium retention, we compared the fluid regulatory responses to 150 min of exercise-induced dehydration, followed by 180 min of *ad libitum* drinking during the follicular and luteal phases of the menstrual cycle, and following treatment with an estrogen/progestin (OC E+P) and a progestin-only (OC P) oral contraceptive in a double-blind, randomized, cross-over design. We found a downward shift in osmotic regulation of arginine vasopressin (AVP) during the luteal phase and OC E+P compared to the follicular phase during exercise. This shift was not accompanied by plasma volume expansion or fluid retention. Fluid and electrolyte losses during exercise were attenuated during the follicular phase of the menstrual cycle, but overall fluid and electrolyte balance was similar across all trials. We also determined that the variability of the fluid regulatory hormones (renin, aldosterone and atrial natriuretic peptide) was high over the course of two menstrual cycles, but slopes and intercepts defining osmotic regulation of AVP were highly reliable. We are in the process of examining the impact of estrogen and progesterone on thermoregulation, although due to our double-blind design, we have no comment on the effects of the oral contraceptives at this point. Preliminary data on 3 women who have completed testing in both menstrual phases reveal no effect of menstrual phase on basal or exercise core or skin temperature, although sweating is attenuated during the luteal phase.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Body fluid regulation, Exercise, Oral contraception, Estrogen, Progestin, Arginine vasopressin, Thermoregulation Defense Women's Health Research Program	57
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

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Ethan R Nadler

Principal Investigator's Signature

October 5 1998

Date

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INTRODUCTION

Methods to regulate and suppress menstruation and provide contraception are needed as women take more active roles in the military. The administration of estrogen and progestin combinations in the form of the oral contraceptive pill has been proposed as a method to regulate menstruation in women during combat and field situations. Alternatively, some contraceptive pills provide progestins only, and contain no estrogen. Combined oral contraceptive pills contain synthetic estrogens, which exhibit 6-10 times the estrogenic activity provided by endogenous, circulating estrogens. Progestin-only pills not only contain no estrogen, but the unopposed progestin tends to down-regulate estrogen receptors. Thus, these two widely used oral contraceptive preparations differ significantly in their estrogenicity. Estrogens have potent effects on the regulation of body water balance (1, 5), so these two forms of oral contraceptive pills may differ in their effects on water regulation, and hence on physical performance under adverse environmental conditions.

Protocol A: Sex Hormone Effects on Body Water Regulation during Dehydration and Rehydration.

Sex hormone administration is accompanied by significant water and sodium retention (1, 5) which leads to plasma volume expansion (3, 4, 55, 63). In fact, variations in plasma volume at rest and during exercise that are observed following estrogen administration and during different phases of the menstrual cycle are comparable to the reported effects of posture, skin temperature and exercise intensity (19). Bilateral oophorectomy results in a 25% loss of blood volume, and replacement of estrogen restores blood volume (17). Oral contraceptive agents, which deliver pharmacological levels of estrogens, increase total body water (5). Fortney et al. (15) demonstrated that estrogen (premarin) administration was associated with an attenuation of the blood volume loss usually associated with bed rest. Some investigators have shown that plasma volume is higher during the follicular phase, when estrogen levels are rising (46, 47).

The mechanism underlying the estrogen-mediated body water retention is unclear, but may be due to alterations in the release of arginine vasopressin (4, 9). No study has addressed the impact of sex hormone administration on body fluid restoration following dehydration, but arginine vasopressin, measured during controlled rehydration, returns to pre-dehydration levels more slowly in women (follicular phase) compared to men (44). This slower restoration of arginine vasopressin is associated with greater fluid retention in women, suggesting the renal response to arginine vasopressin is unaffected by estrogen. These data also suggest a role for estrogen in the recovery of arginine vasopressin following dehydration. Prior to the present experimental series, no studies had evaluated systematically the impact of variable estrogen doses found in oral contraceptive pills on fluid regulation in women.

Our study was designed to test the hypothesis that oral contraceptive pills containing estrogen increase the thirst and arginine vasopressin response to plasma osmolality and plasma volume alterations during progressive dehydration to a greater degree than progestin-only pills. We expected that this increase in osmotic sensitivity

would result in enhanced fluid intake and water retention during a subsequent *ad libitum* rehydration period.

In addition to the changes in arginine vasopressin, plasma concentrations of the sodium and water retention hormones, renin and aldosterone, increase during pregnancy (41), during estrogen-dominant oral contraception (5, 62) and during ovarian stimulation (41). Elevations in plasma estrogen concentration increase sodium retention (1, 43), due either to changes in body sodium distribution (1, 5), renal sodium reabsorption (10), or renin and aldosterone actions (41). During the mid-luteal phase of the menstrual cycle however, PRA and aldosterone increase only when ovulation occurs (30), indicating that a functioning corpus luteum (and the progesterone it secretes) is necessary to augment the renin-angiotensin-aldosterone system. In young, cycling women, the mid-luteal phase increase in endogenous progesterone is accompanied by an increase in estrogen, which may enhance the progesterone effect on the renin-aldosterone system (41).

The impact of estrogen on the renin-aldosterone system and sodium regulation has not been studied during dehydration, a time when both sodium and water retention systems are stimulated. In this study, we used a dehydration-rehydration protocol during combined (estradiol and progestin) or progestin-only oral contraceptive pills in order to distinguish specific estrogen effects on the sodium regulating hormones. The synthetic progestin, norethindrone, does not possess antimineralocorticoid properties (60), and the unopposed progestin down-regulates estrogen receptors. Thus, these two oral contraceptive preparations differ significantly in their estrogenicity, and as such, may differ in their effects on sodium and water regulation. We hypothesized that the estradiol contained in combined oral contraceptive pills would slow the rate of electrolyte loss during dehydrating exercise, and enhance fluid and sodium retention during rehydration relative to control (follicular and luteal phase), and progestin-only pills. We further hypothesized that the greater fluid and sodium retention would be related to an estrogen-mediated stimulation of the renin-aldosterone system.

METHODS

Study design:

Ten women volunteered to participate in the dehydration experiments. Subjects were non-smoking, healthy women, ages 21-31, with no contraindications to oral contraceptive use. All subjects were interviewed about their medical history, and had medical and gynecological examinations before admission to the study. During the month preceding the first dehydration/rehydration exposure, blood volume was determined by Evan's Blue dye dilution (procedures are described below). On the same day, following the blood volume assessment, maximal oxygen consumption ($VO_{2peak,}$) was determined with an automated metabolic cart (Sensor Medics Corp, Yorba Linda, CA). The preliminary tests were all conducted in the follicular phase of the menstrual cycle.

Each woman served as her own control. Upon entering the study, the subjects were assigned (double-blind) to undergo experimental testing after four weeks of either continuous combined (estrogen/progestin) or progestin-only treatment (Fig.1). After

completing the studies on one treatment protocol, subjects crossed over to the other treatment following a 4-week "washout" period.

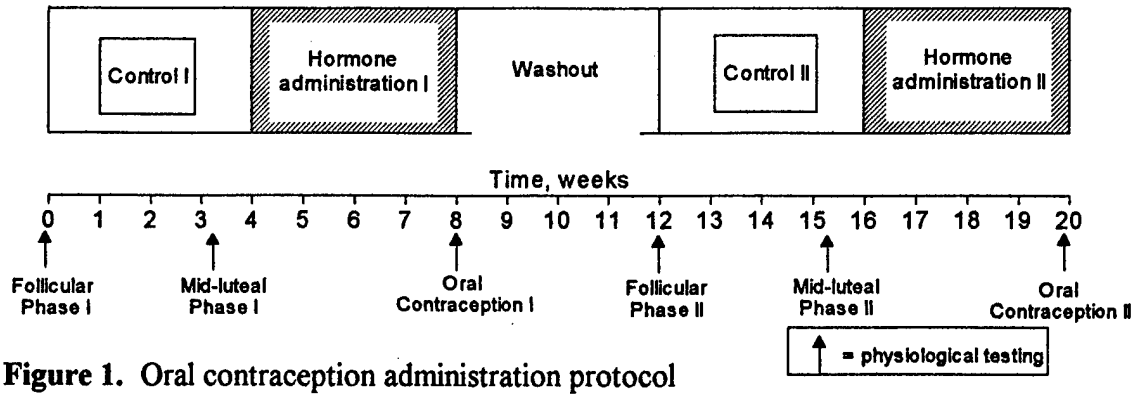

Figure 1. Oral contraception administration protocol

For estrogen/progestin combined treatment (OC E+P), subjects received 0.035 mg of ethinyl estradiol and 1 mg of the progestin norethindrone daily. For progestin-only treatment (OC P), subjects received norethindrone, 1 mg/day. All studies were begun within 2 hours of the daily pill ingestion, when peak serum hormone levels occur (8).

Because sex hormones vary across the menstrual cycle, some variation in the dependent variables over the course of the menstrual cycle may exist. Therefore, the study design employed two dehydration baseline studies, carried out in the early-follicular phase (2-5 days after the beginning of menstrual bleeding) and mid-luteal phase of the menstrual cycle in the month preceding each oral contraception treatment. *The two control tests were completed during the month before each 28-day pill treatment (Fig 1).* The luteal phase was determined individually by the use of ovulation prediction kits (OvuQuick, Quidel Corp, San Diego, CA) that accurately identify the luteinizing hormone peak. To verify phase of the menstrual cycle, plasma levels of estrogen and progesterone were assessed from the control (pre-exercise) blood sample.

Dehydration experiments

Volunteers arrived at the laboratory between 7:00 - 8:00 am, after having eaten only a prescribed low fat breakfast (~ 300 kcal). The subjects refrained from alcohol and caffeine for 12 hours prior to the experiment. Blood volumes were un-manipulated prior to each of the experiments, although subjects were well hydrated by drinking 7 ml/kg body weight of tap water at home before arrival at the laboratory. Upon arriving at the laboratory, the subjects gave a baseline urine sample, were weighed to the nearest 10 g on a beam balance and then sat on the contour chair of a cycle ergometer in the test chamber (27°C, 30% rh) for 60 min of control rest. During the control period, an indwelling catheter was placed in an arm vein. Electrodes and a blood pressure cuff were placed and resting blood pressure (Colin Medical Instruments Corp, Komaki, Japan) and heart rate (EKG) recorded at the end of the 60-min control period. At the end of the control period, a (20 ml) blood sample was drawn, control thirst tests (see below) administered and urine

collected. Hydration state was assessed from the specific gravity of the control (pre-exercise) urine sample (mean = 1.001).

Dehydration protocol

We have modified a Monark cycle ergometer by placement of an adjustable contour seat behind the pedals so that the subject was seated with legs nearly in a horizontal position. The exercise intensity was adjusted by changing the tension on the flywheel, and was normalized to each subject as determined by her individual VO_{2peak} test.

Following the control period, the chamber temperature was increased to 36°C. The subjects exercised at 50% maximal power output without fluids for 150 min, with 5 min rest periods every 25 min. Blood samples (10-20 ml) were drawn immediately prior to the rest periods at 60, 120 and 150 min during exercise. Thirst ratings were also assessed immediately prior to rest periods at 30, 60, 90, 120 and 150 min of cycling. During exercise, sealed absorbent patches (Sudormed, Santa Anna, California) were placed on the thigh, forearm, chest, back and forehead for 20-40 min periods for sweat collection. The sweat patch consisted of 4.7 x 3.1 cm filter paper, sealed and affixed to the skin with tegaderm. The area used for the patch was cleaned with deionized water prior to placement and wiped with a clean dry towel. After sampling, the patches were transferred to plastic screw-capped bottles. Local sweat rate was determined by patch weight increase (to 0.0001 g) from the dry weight per min on the skin. The fluid in the patches was collected by centrifugation with nylon MicroFuge centrifuge filter tubes and analyzed for sodium and potassium concentrations. Heart rate and blood pressure were assessed every 10 min throughout exercise. Body weight was determined at 60, 120 and 150 min of exercise, and urine samples were collected at the end of exercise. At the end of exercise, the chamber temperature was reduced to 27° C for the 3.5 hour recovery period.

Following dehydration, volunteers rested for 30 min in a contour chair without access to fluids to allow the body fluid compartments to stabilize, after which, the subjects drank water *ad libitum* for 180 min. Heart rate and blood pressure were assessed every 10 min throughout stabilization and rehydration. Blood (10 ml) was sampled during the early period of rehydration (just prior to drinking, at 15 min of drinking) and at 30, 60, 120 and 180 min of rehydration (20 ml). Urine samples were collected at each 60 min of rehydration and body weight was measured every 60 min of rehydration.

All blood samples were analyzed for hematocrit, hemoglobin, total protein, osmolality, and the concentrations of creatinine, glucose, urea, sodium, potassium, and arginine vasopressin. The control and final blood samples were analyzed for 17 β-estradiol and progesterone. Blood samples at control, 60, 120 and 150 min of dehydration and at 0, 30, 60, 120 and 180 min of *ad libitum* drinking were also analyzed for the concentration of atrial natriuretic peptide, aldosterone, and plasma renin activity. All urine samples were analyzed for volume, osmolality, and sodium, potassium, and creatinine concentrations.

Blood sampling

All blood sampling was done via a 19 gauge Intracath catheter placed in an arm vein. Subjects were semi-recumbent during placement of the catheter and are seated for 60 min prior to sampling to ensure a steady state in plasma volume and constituents.

Blood samples were separated immediately into aliquots. The first was analyzed for hemoglobin and hematocrit. A second aliquot was transferred to a heparinized tube, and a third aliquot for the determination of serum sodium and potassium concentrations was placed into a tube without anticoagulant. All other aliquots were placed in tubes containing EDTA. The tubes were centrifuged and the plasma taken off the heparinized sample analyzed for sodium, potassium, osmolality, glucose, urea creatinine and aldosterone. The EDTA samples were analyzed for concentrations of arginine vasopressin and atrial natriuretic peptide and plasma renin activity.

Blood volume

Absolute blood volume was measured by dilution of a known amount of Evan's blue dye. This technique involves injection of an accurately determined volume of dye (by weight, since the specific density is 1.0) into an arm vein and taking blood samples for determination of dilution after complete mixing has occurred (10, 20 and 30 min). Plasma volume was determined from the product of the concentration and volume of dye injected divided by the concentration in plasma after mixing, taking into account 1.5% lost from the circulation within the 10 min. Blood volume was calculated from plasma volume and hematocrit concentration corrected for peripheral sampling.

Thirst ratings

The perception of thirst was assessed by asking the subject to make a mark on a line rating scale in response to the question, 'How thirsty do you feel now?' The line is 175 mm in length and is marked 'not at all' on one end and 'extremely thirsty' at the 125 mm point. We tell subjects that they can mark beyond the 'extremely thirsty' point if they wish and may even extend the line if they feel it necessary. This method was developed by Marks et al. (29) and has been used with great success in the evaluation of several sensory systems. We have found an extraordinarily good relationship between the perception of thirst and plasma osmolality during hypertonic saline infusion and dehydration in young volunteers.

Calculations

Total water loss due to dehydration was determined from body weight loss. Net fluid gain during rehydration was calculated by subtracting total urine loss from water intake, assuming that respiratory and sweat losses were negligible in the 27°C recovery condition. Electrolyte losses in sweat and urine during dehydration were calculated by multiplying the volume of water loss by the concentration of electrolyte in each fluid. Whole body sweat electrolyte concentration was calculated from sweat rate, local electrolyte concentration and body surface area using the following equation:

$$[E]_m = (0.07[E]_{fh}SR_{fh} + 0.36[E]_{tr}SR_{tr} + 0.13[E]_{fa}SR_{fa} + 0.32[E]_{th}SR_{th}/ 0.07SR_{fh} + 0.36SR_{tr} + 0.13SR_{fa} + 0.32SR_{th}) \quad (54)$$

where the subscripts m, fh, tr, fa and th are whole body mean, forehead, trunk, forearm and thigh; [E] is electrolyte concentration (sodium or potassium, mEq/l), and SR is local sweat rate ($mg \cdot min^{-1} \cdot cm^{-2}$); and the constants 0.07, 0.36, 0.13 and 0.32 represent the

percent distribution of body surface in the head, trunk, arms and legs, respectively. Total electrolyte loss from sweat was calculated by multiplying $[E]_m$ and total body sweat loss, calculated from the change in body weight during exercise. Electrolyte losses during rehydration were calculated by multiplying the volume of water loss by the concentration of electrolytes in the urine.

Changes in plasma volume were estimated from changes in hemoglobin (Hb) and hematocrit (hct) concentrations from the control (pre-exercise) sample according to the equation:

$$\% \Delta PV = 100\ [[(Hb_b)/(Hb_a)][(1\text{-}hct_a \cdot 10^{-2})]/[(1\text{-}hct_b \cdot 10^{-2})]] - 100$$

where subscripts a and b denote measurements at time a and control, respectively. Hemoglobin is measured in triplicate by the cyanomethemoglobin technique and hematocrit in triplicate by the microhematocrit method.

Fractional excretions of water (FE_{H_2O}) and Na^+ (FE_{Na+}) were calculated from the following equations:

$$FE_{H_2O} = (U_V/GFR) \cdot 100$$

$$FE_{Na+} = (U_V \cdot [Na^+]_u/GFR \cdot [Na^+]_f) \cdot 100$$

$$[Na^+]_f = \text{the Donnan factor for cations } (0.95) \cdot [Na^+]_s$$

where the subscripts f and u are glomerular filtrate and urine respectively, U_v is urine flow rate, and $[Na^+]_s$ is $[Na^+]_s$ in protein-free solution (mEq/kg H_2O). Glomerular filtration rate (GFR) was estimated from creatinine clearance.

Blood analysis:

Plasma, sweat and urine sodium and potassium are measured by flame photometry (Instrumentation Laboratory model 943), plasma osmolality by freezing point depression (Advanced Instruments 3DII), and plasma proteins by refractometry. Plasma glucose, urea and creatinine concentrations are determined by colorimetric assay (Sigma Diagnostic Products). Plasma concentrations of arginine vasopressin, 17 β-estradiol, progesterone, aldosterone, atrial natriuretic peptide, and plasma renin activity were measured by radioimmunoassay. Intra- and inter-assay coefficients of variation for the mid-range standard for AVP (4.52 pg/ml) were 6.0 % and 3.4 % (Immuno Biological Laboratories (IBL), Hamburg, Germany), for 17 β-estradiol (64.3 pg/ml) were 3.7 % and 4.0 % (Diagnostic Products, Los Angeles, CA), for progesterone (3.7 pg/ml) were 2.1 % and 2.5 % (Diagnostic Products), for $P_{[ALD]}$ (132 pg/ml) were 3.4 % and 3.6 % (Diagnostic Products, Los Angeles, CA), for $P_{[ANP]}$ (63.3 pg/ml) were 5.1 % and 5.2 % (Diasorin), and for PRA (4.5 $ng \cdot ml^{-1}$ ANG$\cdot hr^{-1}$) were 2.3 % and 2.9 % (Diasorin, Stillwater Minnesota). The assay for AVP has a sensitivity of 0.8 pg/ml, which is necessary to detect small, but important, changes in this hormone.

RESULTS

Combined oral contraceptive administration caused severe nausea in one woman, and she did not complete dehydration testing while on this pill, so all of her control data for OC E+P have also been excluded. In addition, one subject dropped out for personal

reasons after completing two of the control tests, so all of her data was excluded from the analysis. This analysis compares the dehydration test responses of 9 women on OC P with their two control tests and 8 women on OC E+P with their control tests. There were no other significant adverse effects of oral contraceptive administration in any of the subjects.

Baseline (Pre-exercise). Pre-exercise body weight was similar for both phases of the menstrual cycle, and sex hormone administration (Table 1). Furthermore, $P_{[E_2]}$ and $P_{[P_4]}$ demonstrate that the subjects were tested in the early follicular phase and mid-luteal phase of the menstrual cycle during both trials. Finally, oral contraceptive administration suppressed the endogenous production of 17 β-estradiol and progesterone (Table 1).

Plasma osmolality was lower in both the luteal phase and following one month of OC E+P and OC P compared to the follicular phase (Fig. 2). Plasma glucose and urea concentrations were unaffected by menstrual phase or either oral contraceptive pill, indicating that the lower P_{Osm} was due to lower $S_{[Na+]}$ (Table 2). Pre-exercise $P_{[AVP]}$ and thirst were unaffected by phase of the menstrual cycle or by oral contraceptive administration (Tables 3 & 4). Hematocrit and [Hb] were elevated during the luteal phase (Table 3), indicating a contraction of plasma volume when compared to the follicular phase (-7.8 ± 2.6 %) and QC E+P (-8.0 ± 3.4 %) and OC P (-5.7 ± 1.6 %). Combined oral contraceptive pills increased plasma volume only slightly (3.2 ± 2.1 %) and OC P did not change plasma volume (-2.3 ± 2.5 %) compared to the follicular phase. There was no effect of menstrual phase or oral contraceptive treatment on plasma protein concentration (Table 3).

Basal PRA and $P_{[ALD]}$ were elevated in both luteal phase tests compared to the follicular phase tests and to the OC E+P and OC P tests (Figs 3 & 4, $P < 0.05$). In contrast, $P_{[ANP]}$ was greatest at baseline in the follicular phase tests and in the OC E+P test (Fig. 5). There were no differences between the OC E+P and OC P tests in PRA, $P_{[ALD]}$ or $P_{[ANP]}$ at baseline. Pre-exercise urine flow, GFR, free water and osmolar clearances and renal electrolyte excretion were similar within subjects prior to each exercise test (Tables 5 & 6).

Heart rate and blood pressure were similar at baseline and dehydration within the follicular and luteal phase tests so the combined mean of the two series is given for the baseline values and for the dehydration tests. Baseline heart rate and mean blood pressure were unaffected by menstrual phase or by oral contraceptive treatment (Tables 7A and 7B).

Exercise. At the end of 150 min of exercise at 36°C, the women lost the same amount of body water through sweating in the early follicular phase (1.5 ± 0.2 and 1.5 ± 0.1 kg), the mid-luteal phase tests (1.4 ± 0.1 and 1.4 ± 0.1 kg), the OC E+P test (1.5 ± 0.1 kg) and the OC P test (1.3 ± 0.1 kg). Heart rate increased to similar levels during dehydrating exercise in the follicular and luteal phase tests and during the OC P test, but this increase was attenuated during the OC E+P test (Tables 7A and 7B). Mean blood pressure did not change during dehydration in any of the experimental conditions.

Exercise increased P_{Osm} and $P_{[AVP]}$, and decreased plasma volume similarly during the follicular and luteal phases, and during OC E+P and OC P (Fig. 2 & Table 3). Linear regression analysis of the individual subjects' data during dehydration indicated significant

correlations between $P_{[AVP]}$ and P_{Osm}, with r values ranging from 0.82 to 0.98. The abscissal-intercepts of the linear $P_{[AVP]}$-P_{Osm} relationship, or "theoretical osmotic threshold" for AVP release, was significantly lower in the mid-luteal phase and OC E+P than in the follicular phase (Table 1, $P < 0.05$). The slopes of this relationship were unaffected by menstrual phase or oral contraceptive pills. Figure 6 shows the downward shift in the linear $P_{[AVP]}$-P_{Osm} relationships during OC E+P and when $P_{[E_2]}$ and $P_{[P_4]}$ were increased during the luteal phase.

The data in Table 4 indicate that thirst increased similarly during dehydration in all conditions. Linear regression analysis of the individual subjects' P_{Osm} and thirst responses indicated significant correlations, with r values ranging from 0.73 to 0.99. Osmotic thirst stimulation was unaffected by menstrual phase and there were no effects of oral contraceptives on the slope or abscissal intercept of this relationship (Table 1).

Plasma renin activity, $P_{[ALD]}$ and $P_{[ANP]}$ increased during exercise in all conditions, with luteal phase values for PRA and $P_{[ALD]}$ remaining above the follicular phase, OC E+P and OC P (Figs. 3 & 4). For $P_{[ANP]}$, neither menstrual phase nor oral contraceptive treatment affected the magnitude of the exercise-induced increases (Fig. 5). Sweat sodium loss was greatest during exercise in the follicular phase tests (56.3 ± 7.0 and 59.4 ± 9.2 mEq, $P < 0.05$), but was similar between the luteal phase tests (45.2 ± 9.1 and 46.5 ± 7.8 mEq) compared to the OC E+P (47.1 ± 10.7 mEq) or OC P (46.7 ± 8.8 mEq) tests. Sweat potassium loss was unaffected by menstrual phase or oral contraception administration (5.32 ±0.71, 5.92 ± 0.59 and 5.35 ± 0.42 mEq for follicular and luteal phase tests and the OC E+P test, respectively) and (5.42 ± 0.57, 4.47 ± 0.39 and 4.86 ± 0.62, for follicular and luteal phase tests, and the OC P test, respectively). Renal sodium excretion was increased during exercise in all conditions, and this increase was greatest during the follicular phase tests (Table 6, $P < 0.05$). The cumulative sodium (sweat + urine) loss was greatest during the follicular phase tests compared to the luteal phase tests, and the OC E+P and OC P tests (Fig. 7).

Rehydration. Ad libitum fluid intake was similar by the end of the 180 min of rehydration on all six experimental test days (Fig 8). At 180 min of *ad libitum* drinking, subjects had restored 41 ± 5 and 40 ± 10 % (follicular phase), 42 ± 7 and 39 ± 6 % (luteal phase), 38 ± 11 % (OC E+P) and 39 ± 7 % (OC P) lost during dehydration. Plasma osmolality was higher throughout the rehydration period in the follicular phase compared to the luteal phase, OC E+P and OC P tests (Fig. 2). Recovery of $P_{[AVP]}$ and thirst was rapid following the beginning of *ad libitum* drinking, and similar during all rehydration tests (Tables 3 & 4).

For the entire rehydration period, area under the curve for PRA (Fig. 3) was lower during the follicular phase tests ($P < 0.05$) compared to the luteal phase tests and the OC E+P test. Area under the curve for $P_{[ALD]}$ (Fig. 4) was significantly greater in the luteal phase tests compared to the follicular phase tests, and compared to the OC P test ($P < 0.05$). There were no effects of oral contraceptives or menstrual phase on $P_{[ANP]}$ during rehydration (Fig. 5).

Urine flow and renal free water clearance were lower at the end of drinking during OC E+P than in both the follicular and the luteal phase tests (Table 6, $P < 0.05$). Cumulative urine loss was greatest (Fig 8, $P < 0.05$) during the follicular phase relative to the

other conditions, although overall fluid balance (i.e. fluid intake - urine output) was unaffected by either phase of the menstrual cycle or oral contraceptive administration. During rehydration, electrolyte excretion was unaffected by menstrual phase or oral contraceptive administration (Table 6). However, because of the greater exercise sodium excretion during the follicular phase, cumulative sodium loss (exercise + rehydration) was greatest during the follicular phase (Fig. 7).

DISCUSSION

Osmotic regulation of AVP and fluid balance

We found that normally cycling young women have a reduction in the osmotic threshold for AVP release during the mid-luteal phase of the menstrual cycle (i.e. when estrogen and progesterone peak). Further, the osmotic threshold for AVP release is lowered during administration of oral contraceptives containing estrogen, but this reduction in threshold did not occur during progestin-only oral contraceptive use. Previously it was demonstrated that estrogen and progesterone upregulate thirst and AVP responses to an osmotic drive (14, 59), but the upregulation could not be attributed to specific estrogen or progesterone effects. Our data extend these early findings by demonstrating a reduction in the P_{Osm} threshold for AVP release during estrogen-containing oral contraception administration. This threshold shift did not occur when the oral contraceptive contained only progestin, implicating estrogen as the hormone mediating the changes in AVP regulation. Because the water intake during the rehydration phase was similar in all our studies, regardless of menstrual phase or oral contraceptive treatment, we are able to conclude that an elevated circulating estrogen alters the body tonicity around which the body regulates fluids.

Estrogen most likely modulates osmotic AVP regulation via its action within the central nervous system, due to the fact that it readily crosses the blood-brain barrier. Studies in lower animals have demonstrated that estrogen acts directly on estrogen-binding neurons in the hypothalamus (2, 4, 12, 37), thereby affecting synthesis and release of AVP. Estradiol receptors have been identified in the nuclei of neurophysin- and AVP-producing cells in the mouse supraoptic nucleus (37), and osmotic stimulation of vasopressinergic neuronal activity is upregulated by estrogen in the supraoptic nucleus of brain slices of ovariectomized rats (4). Estrogen may also modulate hypothalamic AVP release indirectly through catecholaminergic (21) and/or angiotensinergic (50) neurons, which bind estrogen and project to the paraventricular and supraoptic nuclei. Using [^{3}H]-labeled estradiol, Heritage et al. (21) identified estradiol binding sites in the nuclei of catecholamine neuronal systems, as well as the presence of catecholamine nerve terminals surrounding estradiol target sites in the paraventricular and supraoptic nuclei. Crowley et al. (13) noted parallel changes in brain norepinephrine and AVP in normally cycling rats, and that ovarian steroids modulated norepinephrine turnover in the paraventricular nucleus, indicating that estrogen may act on the osmoregulatory system through catecholamines. There also is evidence for cholinergic and angiotensinergic innervation of

vasopressinergic cells in the paraventricular and supraoptic nuclei, both of which are modulated by sex steroids (50).

Peripheral mechanisms for the estrogen effect on osmotic stimulation of AVP are unlikely to participate in the response. For example, plasma volume reduction, such as that which occurred during the mid-luteal phase, could have contributed to the lower P_{Osm} threshold for AVP release because plasma volume is a potent AVP stimulus. However, this mechanism seems unlikely because the luteal phase-plasma volume contraction was not associated with a fall in blood pressure. Further, AVP was also upregulated during OC E+P administration, during which changes in pre-dehydration plasma volume did not occur. Atrial natriuretic peptide has also been shown to suppress the osmotically-induced rise in AVP (11) but the follicular phase and OC E+P were both associated with greater plasma atrial natriuretic peptide levels (45), and had vastly different vasopressin responses.

Despite the lower osmotic threshold for AVP, there were no changes in water intake, which matched urine output, indicating a new set point for fluid regulation in the presence of high plasma estrogen levels. In addition to reducing the osmotic threshold for AVP release, estrogen may alter the renal sensitivity to AVP by attenuating its antidiuretic action. There is evidence that estrogen modulates AVP action in the rat collecting duct (13) at the receptor level (52). Our observation that the greater osmotic secretion of AVP in the mid-luteal phase of the menstrual cycle was not accompanied by increased water retention is consistent with these findings. In contrast, we also found that renal C_{H_2O} was reduced during combined estrogen and progesterone administration despite similar $P_{[AVP]}$. Moreover, estrogen administration to postmenopausal women has been shown to increase renal concentrating response (U_{Osm}/P_{Osm}) to hypertonic saline infusion, despite similar $P_{[AVP]}$ responses (43). Future studies that determine the renal dose-response relationship of AVP are necessary to determine the impact of estrogen and progesterone on the kidney.

Finally, combined estrogen and progestin oral contraception administration increased plasma volume by as much as 12.4 % relative to the mid-luteal phase of the menstrual cycle. Estrogen-mediated increases in plasma volume are consistent with earlier findings in postmenopausal (1, 43) and young women (5, 15). The estrogen-mediated plasma volume expansion is not always accompanied by changes in water retention, and the mid-luteal phase plasma volume contraction not always associated with greater urine loss. A number of earlier studies demonstrated that high plasma levels of estrogen and progesterone alter Starling forces to favor protein and fluid movement out of the vasculature (25, 26, 56, 57). Therefore, these steroids may have their primary effect by altering body water distribution, rather than body water balance.

Sodium Regulation

Our experimental design enabled us to isolate estrogen effects on the renin-aldosterone system because norethindrone administered alone and with estradiol did not exhibit antimineralocorticoid properties. Our major finding was that neither estrogen dominant, nor progestin-only oral contraceptives increased PRA or $P_{[ALD]}$; rather, we found only the high endogenous estrogen and progesterone present in the luteal phase

enhanced PRA and $P_{[ALD]}$. Sodium loss (sweat + urine) was attenuated during dehydration in the luteal phase and during OC E+P and OC P, but these losses were not necessarily associated with increases in the sodium regulation hormones indicating that norethindrone inhibits sodium loss, but through a mechanism other than the renin-aldosterone system.

Combined oral contraceptive pills deliver pharmacological levels of ethinyl estradiol (7), which is almost identical in structure to the most biologically active form of endogenous estrogen, 17 β-estradiol, although with four times the potency (28). Our data do not support a role for estrogen in the stimulation of the renin-aldosterone system because OC E+P did not augment renin or aldosterone. Norethindrone, a progestational derivative of testosterone, differs in structure from endogenous progesterone. Endogenous progesterone inhibits aldosterone-dependent sodium reabsorption at distal sites in the nephron and produces a transient natriuresis (33) followed by a compensatory stimulation of the renin-aldosterone system (30, 53, 62). In contrast, norethinedrone does not possess antimineralocorticoid properties because neither OC E+P nor OC P led to increases in PRA or $P_{[ALD]}$. Nonetheless, administration of norethindrone, with and without estrogen, enhanced sodium retention, suggesting this synthetic form of progesterone may act directly on the renal tubules.

Our data extend earlier findings demonstrating plasma volume contraction concomitant with enhanced PRA and $P_{[ALD]}$ during the mid-luteal phase of the menstrual cycle at rest, exercise and heat exposure (47, 49). The luteal phase was characterized by a baseline plasma volume contraction of ~220 ml compared to the follicular phase and of ~283 ml compared to OC E+P. Basal plasma sodium content also decreased in the luteal phase (377 ± 22, 340 ± 26, 388 ± 24, 368 ± 22 mEq, $P < 0.05$, for the follicular and luteal phases, and OC E+P and OC P, respectively). However, although plasma volume and sodium content contraction are powerful stimuli to the renin-aldosterone system, they were not accompanied by changes in blood pressure so may not have contributed directly to the increases in PRA and $P_{[ALD]}$.

Progesterone and/or estrogen may modulate plasma volume and sodium content through inhibition of ANP release from cardiac myocytes. Atrial natriuretic peptide plays a role in the homeostatic feed back system that regulates sodium balance, that is, sodium- and volume-retaining stimuli increase ANP, which, in turn, antagonizes renin and aldosterone (7, 34, 42). Progesterone administration can suppress $P_{[ANP]}$ (61), so increases in circulating endogenous progesterone may inhibit ANP release during the luteal phase, and thus reduce sodium excretion. Furthermore, there is evidence that progesterone interferes with the inhibitory effects of ANP on aldosterone secretion (32, 34), suggesting that progesterone may enhance $P_{[ALD]}$ not only by attenuating ANP release, but by reducing the inhibitory actions of $P_{[ANP]}$ on the adrenal cortex. In our investigation, combined oral contraceptive pills increased $P_{[ANP]}$ at baseline and during dehydration, while OC P reduced $P_{[ANP]}$ to luteal phase levels, suggesting the estrogen in OC E+P may have modified a progesterone-modulated $P_{[ANP]}$ inhibition during dehydration. Alternatively, estrogen receptors are found in cardiac myocytes (51), so estradiol may stimulate ANP release directly.

Our findings also suggest that estrogen impacts water and protein distribution in the body. Despite the plasma volume contraction in the luteal phase, total protein concentrations were unchanged during the luteal phase tests, indicating that both water

and protein left the vasculature. Indeed, circulating plasma proteins (183.7 ± 11.3, 175.6 ± 10.7, 192.6 ± 13.1 and 184.8 ± 10.6 g, combined means for the follicular and luteal phase tests, and OC E+P and OC P, respectively) were lowest in the luteal phase tests compared to all other test conditions. Estrogen-mediated changes in body water and protein distribution are consistent with earlier studies in which the level of plasma volume expansion could not account for level of increases in overall body water retention (43). For example, during hypertonic saline infusion in estrogen-treated postmenopausal women, body water retention was increased by 31%, but plasma volume was unchanged (43). Finally, earlier studies have demonstrated that estrogen and/or progesterone alter transcapillary fluid dynamics to favor fluid and protein movement into the extravascular (interstitial) compartment (56, 57).

Any estrogen- or progesterone- mediated changes in transcapillary fluid dynamics may also have occurred via ANP. Atrial natriuretic peptide has important effects on body fluid dynamics, and may contribute to plasma volume regulation by inducing extravascularization (18, 36). Low-dose ANP infusions (to ~150 pg/ml) augment the capillary filtration coefficient (18), probably due to ANP-mediated changes in protein permeability. The increase in plasma protein permeability allows plasma proteins to escape from the circulation into the interstitial fluid, decreasing the rise in the colloid osmotic pressure of the microvasculature, opposing fluid reabsorption from the interstitium, and thus causing the extravascular efflux of proteins and fluid. Although estrogen and progesterone may increase ANP release, or impact its actions, the extent to which these hormones interact with ANP and modulate body water distribution has not been determined.

We used oral contraceptive pills to evaluate estrogen effects on the renin-aldosterone system and sodium regulation during dehydration and a subsequent rehydration period. During dehydration, we found that sodium loss was attenuated during the luteal phase and during administration of oral contraceptives containing estradiol and progestin, but these effects on sodium regulation were not mediated through the renin-aldosterone system. While estrogen does not appear to have direct effects on the renin-angiotensin-aldosterone system, this hormone may impact sodium regulation by modifying a progesterone-modulated inhibition of ANP release. In addition, the changes in sodium regulation may also have been influenced by the changes in resting plasma volume and sodium content.

CONCLUSIONS

We found that normally cycling young women have a reduction in the osmotic threshold for AVP release during the mid-luteal phase of the menstrual cycle (i.e. when estrogen and progesterone peak). Further, the osmotic threshold for AVP release is lowered during administration of oral contraceptives containing estrogen, but this reduction in threshold did not occur during progestin-only oral contraceptive use. Previously it was demonstrated that estrogen and progesterone upregulate thirst and AVP responses to an osmotic drive (14, 59), but the upregulation could not be attributed to specific estrogen or progesterone effects. Our data extend these early findings by demonstrating a reduction in the P_{Osm} threshold for AVP release during estrogen-containing oral contraception administration. This threshold shift did not occur when the

oral contraceptive contained only progestin, implicating estrogen as the hormone mediating the changes in AVP regulation. Because the water intake during the rehydration phase was similar in all our studies, regardless of menstrual phase or oral contraceptive treatment, we are able to conclude that an elevated circulating estrogen alters the body tonicity around which the body regulates fluids.

Regarding sodium regulation, we used oral contraceptive pills to evaluate estrogen effects on the renin-aldosterone system and sodium regulation. During dehydration, we found that sodium loss was attenuated during the luteal phase and during administration of oral contraceptives containing estradiol and progestin, but these effects on sodium regulation were not mediated through the renin-aldosterone system. While estrogen does not appear to have direct effects on the renin-angiotensin-aldosterone system, this hormone may impact sodium regulation by modifying a progesterone-modulated inhibition of ANP release. In addition, the changes in sodium regulation may also have been influenced by the changes in resting plasma volume and sodium content.

Protocol A: Reliability of fluid regulation hormones

INTRODUCTION

Despite the continued study of changes in the fluid and sodium regulating hormones, there were no studies examining their stability, or reliability within a given phase, over the course of two or more menstrual cycles. Differences in reported plasma concentrations of these hormones across different menstrual cycles can be affected by natural variations within a woman, by inaccurately choosing the appropriate day of each phase of the menstrual cycle to conduct physiological testing, by differences in water and/or sodium intake, or by difficulty with the hormone analysis techniques. We determined the reliability of the fluid and sodium regulating hormones, aldosterone, renin, arginine vasopressin and atrial natriuretic peptide, at rest and in response to dehydrating exercise over two menstrual cycles. Accordingly, we tested the reliability of the fluid regulating hormones in our subjects on the above-described dehydration testing days: twice during the early follicular phase (when estrogen and progesterone are low) and twice during the mid-luteal phase of the menstrual cycle (when estrogen and progesterone are high).

METHODS

Subjects were nine non-smoking, healthy women, ages 21-3. All subjects were interviewed about their medical history, and had medical and gynecological examinations before admission to the study. During the month preceding the first dehydration/rehydration exposure maximal oxygen consumption ($VO_{2peak,}$) was determined with an automated metabolic cart (Sensor Medics Corp, Yorba Linda, CA). This preliminary test was conducted in the early-follicular phase of the menstrual cycle.

The study design employed four dehydration experiments, two conducted in the early-follicular phase (2-4 days (3 ± 1days) after the beginning of menstrual bleeding) and two in the mid-luteal phase of the menstrual cycle (20-25 days (22 ± 2 days) after the start of menstrual bleeding). For the mid-luteal phase tests, the subjects were tested between days 7-10 following the LH peak, and therefore approximately 6-9 days after ovulation. The dehydration protocol is described on pages 3-5 of this Progress Report (See "Dehydration experiments").

Statistical Analysis. Pearson's Product Moment Correlation on individual data was used to assess the slope and abscissal intercepts of the P_{Osm} - $P_{[AVP]}$ relationship during dehydration (14). The within-phase reliability of our most important dependent variables, fluid regulating hormones and osmotic regulation of AVP, measured at rest, dehydration and rehydration, was determined with Cronbach's α, assuming a value ≥ 0.80 as a acceptable level of reliability (6). Areas under the curve (AUC, trapezoid method) were calculated during the rehydration period for PRA, $P_{[ALD]}$ and $P_{[ANP]}$, and their reliability determined within a given menstrual phase using Cronbach's α. We used repeated measures ANOVA models, followed by Bonferoni's *t*, to test differences in the dependent variables

within menstrual phases. Data were analyzed using BMDP statistical software (BMDP Statistical Software, Inc., Los Angeles, CA), and expressed as mean ± SEM.

RESULTS

Within-phase reliability.

Early follicular phase. Within the follicular phase, there were no significant differences between the means of any of the variables during rest, dehydration and rehydration (Table 8). However, with the exception of $P_{[ANP]}$, none of the resting values of the fluid regulating hormones attained sufficiently high Cronbach's α to be considered reliable (Table 9). Reliability was improved following dehydrating exercise for $P_{[AVP]}$ and PRA; although it remained low for $P_{[ALD]}$ ($\alpha = 0.66$) and remained high for $P_{ANP]}$ ($\alpha = 0.90$). During dehydration, both the slope and abscissal intercept of the P_{Osm} - $P_{[AVP]}$ relationship were highly reliable within the follicular phase, attaining Cronbach's α of 0.96 and 0.90, respectively. Again, $P_{[AVP]}$, $P_{[ALD]}$ and PRA were not reliably reproduced during rehydration, while Cronbach's α for $P_{[ANP]}$ was 0.93. Plasma estrogen concentration was highly reproducible within the follicular phase tests, attaining Cronbach's α of 0.85, but $P_{[P_4]}$ attained a Cronbach's α value of only 0.62 between tests in the follicular phase.

Mid-luteal phase. Similar to the follicular phase, there were no differences mean hormonal concentrations at rest, after dehydration or during rehydration within the mid-luteal phase (Table 8). Again, resting values for $P_{[AVP]}$, $P_{[ALD]}$ and PRA were not highly reproducible between the two mid-luteal phase tests (Table 9). Reliability for $P_{[ANP]}$ was greater compared to the other fluid regulating hormones, at rest and during exercise and rehydration, and again, despite high levels of reliability for osmotic regulation of AVP (Table 9), resting and rehydration levels of $P_{[AVP]}$ were not consistently correlated within the luteal phase tests. In contrast to the follicular phase however, both $P_{[E_2]}$ and $P_{[P_4]}$ were highly reliable between the two luteal phase tests, yielding Cronbach's α values of 0.93 and 0.93, respectively.

CONCLUSIONS

We examined the within-phase reliability of plasma concentration of fluid and sodium regulating hormone concentrations between two separate menstrual cycles at rest and in response to dehydration during the early follicular and mid-luteal phases. Resting and recovery plasma concentrations of AVP, aldosterone and PRA were not reproducible within each of the different menstrual phases; however, there were no statistical differences between the means of any of these hormone concentrations indicating that the within-subject inconsistency remains undetected when only the means are tested or reported. Nonetheless, because between phase differences in the hormone concentrations far exceed the variability within the phases, the low within-phase reliability does not

prevent the detection of menstrual phase-related changes in these variables. In contrast to rest, however, $P_{[AVP]}$, PRA and $P_{[ANP]}$ responses to dehydrating exercise were highly reliable within each menstrual phase indicating that hormonal responses to stress are more consistent in spite of the variability in baseline values.

Responses to technical issues regarding the Progress Report from 1997:

(1) We have attempted to clarify the timing the experiments in the text (Page 3) with the following *"Because sex hormones vary across the menstrual cycle, some variation in the dependent variables over the course of the menstrual cycle may exist. Therefore, the study design employed two dehydration baseline studies, carried out in the early-follicular phase (2-5 days after the beginning of menstrual bleeding) and mid-luteal phase of the menstrual cycle in the month preceding each oral contraception treatment."* In addition, we have added a figure to illustrate the protocol (See Figure 1).

(2) The letter "h" was changed to "hours" after the "2" in this sentence to clarify the timing on page three. The sentence now reads "All studies were begun within 2 hours of the daily pill ingestion when peak serum hormone levels occur (8)."

(3) The other major technical issue raised by the reviewers was regarding the long-term effect on these hormones on the variables we have measured. Of course we cannot answer this question from our data because our treatment only extends one month (28 days). However, there are a few studies that demonstrate that the effects on body water expansion (5), and body water distribution (56, 57) last least as long as 6 months (56, 57) to one year (5). It has also been demonstrated that 2 months of oral contraceptive treatment leads to increases in blood volume at rest, as well as increases in stroke volume and cardiac output during exercise (27). These studies suggest that the changes in body fluid regulation are likely long-term, and may continue until estrogen and/or progesterone administration is stopped.

Protocol B: Sex Hormone Effects on Thermoregulation during Exercise in the Heat.

The regulation of body temperature in humans is known to interact with systems that regulate volume and osmotic pressure of the extracellular fluid (31). Blood volume expansion improves the efficiency of cardiovascular and thermoregulatory responses during physical activity. In a study in which we manipulated blood volume in young men by ~ 9% of normal (16), after 30 min of moderately heavy exercise in the heat, internal temperature rose to 38.6°C in the control condition, to 38.3°C in the volume-expanded condition and to 38.9°C in the volume-contracted condition. The likely reason for the dependence of heat transfer on absolute blood volume during exercise in the heat is that the ability of the heart to pump blood to the skin, and therefore provide increased convective heat transfer from the body core to the skin, is a function of preload. When blood volume is expanded, cardiac stroke volume increases, resulting in elevated cardiac output and improved ability to deliver blood to muscle and skin simultaneously, where heat transfer takes place. Conversely, blood volume contraction results in a gradual fall in preload during exercise (58), a reduction in cardiac output and an associated increase in skin vascular resistance at any internal temperature, explaining the decrease in heat transfer. These observations imply that a reflex sensitive to changes in the filling pressure of the heart influences the distribution of blood flow (and thus the resident blood volume) and thereby affects the body's ability to dissipate the excess heat produced during exercise.

The temperature threshold for the onset of a thermoregulatory effector response, i.e. sweating and peripheral vasodilation, is defined as the core temperature above which the effector response is greater than that of baseline. A shift in the core temperature threshold is often referred to as a change in the set-point for temperature regulation (48). A reduction in the set-point for temperature regulation secondary to blood volume expansion has profound effects on performance of physical activity in the heat because core temperature is maintained at a lower level and strain on the cardiovascular system is reduced. Conversely, dehydration (plasma volume loss) elevates exercise core temperature (38, 40) and decreases exercise tolerance (39, 40).

Estrogen may alter the threshold for thermoregulation during exercise in the heat. Core temperature responses to passive heating and exercise in heat are reduced during the follicular phase of the menstrual cycle, the cycle phase characterized by rising estrogen levels (22-24, 35). Haslag and Hertzman (20) demonstrated that the onset of thermoregulatory sweating during whole body heating occurred at a lower core temperature in women during their follicular phase. Stephenson and Kolka reported lower core temperature thresholds of both sweating (24, 46) and cutaneous vasodilation in a hot environment (46) during the follicular phase. No studies assess directly the effect of oral contraceptives on thermoregulatory responses during exercise in the heat, but the thresholds for the onset of sweating and vasodilation were reduced by 0.47°C and 0.48°C, respectively following 2 weeks of estrogen replacement therapy in postmenopausal women (55). The plasma volume expansion that is an outcome of estrogen administration (55), and the follicular phase of the menstrual cycle (47) may play an important role in the improved thermoregulation in the presence of high plasma levels of estrogen. This phase of the study is designed to determine the impact of estrogen-induced plasma volume expansion on thermoregulatory responses to exercise in the heat.

METHODS

Study design:

The primary goal of these experiments is to determine the effect of hormone manipulation on thermoregulation in young women. As of this date, eight women have volunteered to participate in the temperature regulation experimental series. Subjects are non smoking, healthy women, ages 21-28, with no contraindications to oral contraceptive use. All subjects are interviewed about their medical history, and have medical and gynecological examinations before admission to the study. During the month preceding the first dehydration/rehydration exposure, blood volume is determined by Evan's Blue dye dilution (procedures are described on page 5 of this progress report). On the same day, following the blood volume assessment, maximal oxygen consumption ($VO_{2peak,}$) is determined with an automated metabolic cart (Sensor Medics Corp, Yorba Linda, CA). The preliminary tests are all conducted in the early follicular phase of the menstrual cycle.

Each woman serves as her own control. Upon entering the study, the subjects are assigned (double-blind) to undergo experimental testing after four weeks of either continuous combined (estrogen/progestin) or progestin-only treatment. After completing the studies on one treatment protocol, subjects cross over to the other treatment following a 4 week "washout" period (Fig 1, page 3). For estrogen/progestin combined treatment, subjects receive 0.035 mg of ethinyl estradiol and 1 mg of the progestin norethindrone daily. For progestin only treatment, subjects receive norethindrone, 1 mg/day. All studies are begun within 2 hours of the daily pill ingestion, when peak serum hormone levels occur (8).

Because sex hormones vary across the menstrual cycle, some variation in the dependent variables over the course of the menstrual cycle may exist. Therefore, the study design employs two heat stress test baseline studies, carried out in the early-follicular phase (2-5 days after the beginning of menstrual bleeding) and mid-luteal phase of the menstrual cycle. The two control tests were completed during the month before each 28 day pill treatment (Fig 1, page 3). The luteal phase is determined individually by the use of ovulation prediction kits (OvuQuick, Quidel Corp, San Diego, CA) that accurately identify the luteinizing hormone peak. To verify phase of the menstrual cycle, plasma levels of estrogen and progesterone are assessed from the control (pre-exercise) blood sample.

Heat Stress Tests

The subjects arrive at the laboratory between 7:00 and 9:00 am, after eating a light (~ 300 kcal) breakfast. Upon reporting to the laboratory, the subjects are weighed to the nearest 10 g on a beam balance, provide a baseline urine sample, are instrumented for the measurement of cardiac output (Cardiac Impedence) and then sit on the contour chair (semi-recumbent) of a cycle ergometer in the test chamber set at 27°C, 30% rh. After being seated, a 20 gauge Teflon catheter is placed in an antecubital or forearm vein and the subject instrumented for the measurement of esophageal and skin temperatures, sweat rate, and blood pressure. Once instrumentation is completed, the subject sits quietly for

20 min of control rest, as the end of which a control blood sample is taken. Baseline arterial blood pressure, cardiac output, esophageal and skin temperatures recorded during the 20 min of control. At the end of the control period, the chamber temperature is increased to 35°C and the subject sits quietly for 20 min of passive heating. Again, arterial blood pressure, cardiac output, esophageal and skin temperatures recorded during the 20 min of passive heating, and a blood sample drawn at the end.

Following passive heating, each woman exercises on the cycle ergometer in the semi-recumbent position for 40 min in a 35°C environment at an estimated exercise intensity of 65% VO_2 max. Measurements are made of arterial blood pressure every 10 min, sweating rate, esophageal temperature and mean skin temperature continuously, and sweat composition at 40 min of exercise. Blood samples (5 & 8 ml) are sampled 5 times during the protocol (total blood drawn is ~ 40 ml) and analyzed for volume and composition changes during the exercise bout. Heart rate is monitored continuously and cardiac output estimates are obtained at baseline, during passive heating and at 10-20 and 30-40 min during exercise. Metabolic rate is measured at control, during passive heating and at 10-15 and 30-35 min during exercise. Body weight is measured at the end of exercise, followed by a final urine collection.

All blood samples are analyzed for hematocrit, hemoglobin, total protein, osmolality, and the concentrations of sodium and potassium. The control blood sample is also analyzed for 17 β-estradiol and progesterone. Blood samples at control, and at 0, 15, 25 and 40 min of exercise are also analyzed for the concentration of aldosterone. Urine is analyzed for volume, osmolality, sodium, and potassium.

RESULTS (preliminary)

As of this date (10/1/98), 2 subjects have completed experiments with "OC A" and 2 have completed experiments with "OC B." One subject has completed two of the control experiments and is currently taking OC B. The other three subjects are in the process of orientation and beginning control heat stress tests. In this report, we will only describe data pertaining to menstrual cycle effects on temperature regulation, for which we have complete data on three subjects. Therefore, the data contained in this report are only preliminary; we have not run statistical analyses because we have do not have complete data on any subjects and hope to avoid premature conclusions and future bias. We expect to complete all data collection by the end of June 1999.

Subject Characteristics

Body weight before exercise appears unaffected by menstrual phase, and was 54.1 ± 3.5 and 54.4 ± 3.6, for the follicular and luteal phases respectively. Control heart rate, stroke volume, cardiac output and blood pressure were similar during the follicular and luteal phases (Table 10).

Effects of menstrual cycle phase on temperature regulation

Baseline

Basal temperature

Baseline core (36.9 ± 0.2°C and 37.0 ± 0.5°C) and skin (31.4 ± 0.5°C and 31.3 ± 0.6°C for follicular and luteal phases, respectively) temperatures were similar between menstrual phases (Fig 9).

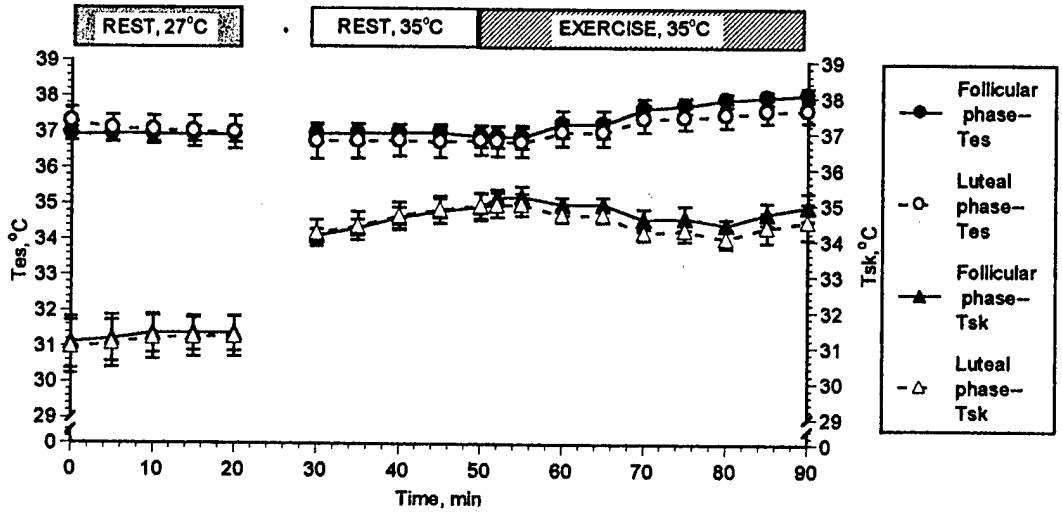

Figure 9. Esophageal (T_{es}) and weighted skin temperature (T_{sk}) during control (27°C), passive heating (35°C) and exercise in the heat (35°C).

Cardiovascular variables

Heart rate, stroke volume and blood pressure were similar during the 20-min control period over the two menstrual phases (Table 10). Heart rate, stroke volume and blood pressure were similar prior to heating and exercise over the two menstrual phases.

Blood components.

Table 11 shows very little change in blood components at rest over the course of the menstrual cycle. The assays for determination of plasma aldosterone, estradiol and progesterone concentrations have not yet been performed.

Passive Heating

Core (esophageal) and skin temperature responses.

Core temperature and T_{sk} were unaffected by menstrual phase (Fig 9). In addition, menstrual phase did not alter sweating rate during passive heating (Fig. 10).

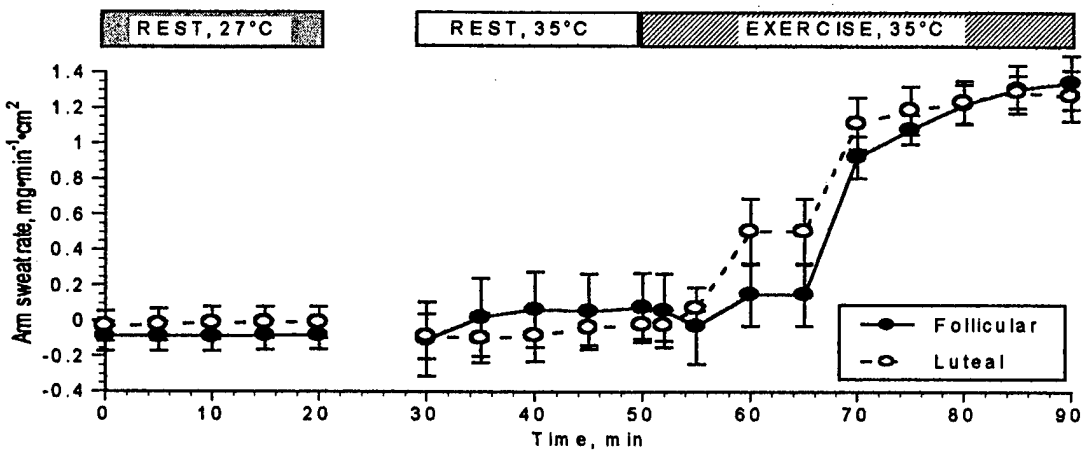

Figure 10. Arm sweat rate during control (27°C), passive heating (35°C) and exercise in the heat (35°C).

Cardiovascular variables

Heart rate, stroke volume and blood pressure responded similarly to passive heating during the follicular and luteal phases (Table 10).

Blood components.

Table 11 shows very little change in blood components during passive heating over the course of the menstrual cycle. The assays for determination of plasma aldosterone, estradiol and progesterone concentrations have not yet been performed.

Exercise responses.

Temperature regulation

Core (T_{es})and skin (T_{sk}) temperatures and were unaffected by menstrual phase. However, there was a greater sweating response for a given temperature increase during the luteal versus the follicular phase (Fig. 11).

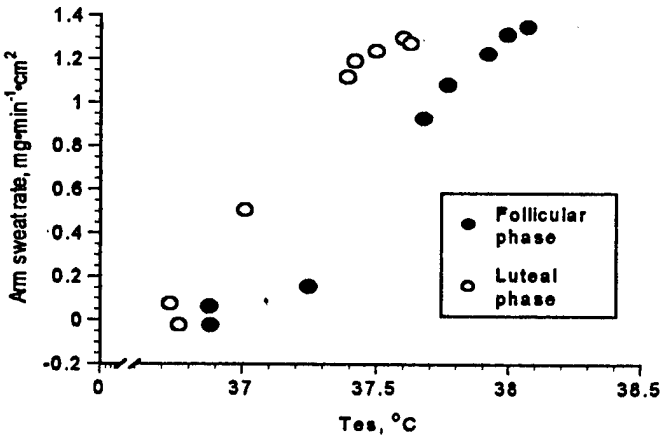

Figure 11. Arm sweat rate as a function of temperature changes during exercise at 35°C.

Cardiovascular variables

Exercise increased heart rate, stroke volume and cardiac output similarly during the follicular and luteal phases. Systolic blood pressure increased slightly, but diastolic decreased simultaneously so there was no overall effect on mean arterial pressure.

Body water and electrolyte loss.

At the end of 40 min of exercise at 35°C, the women lost 0.6 ± 0.3 and 0.5 ± 0.1 kg through sweating during the follicular and luteal phases respectively. At the end of exercise, urine volume was lower during the follicular phase (183 ± 35 ml) compared to the luteal phase (101 ± 26 ml). However, renal sodium (17. ± 5.3 versus 78.9 ± 20.7 mEq) and potassium (12.5 ± 4.3 versus 49.1 ± 8.7 mEq, for follicular and luteal phases respectively) were greater in the luteal phase. Sweat sodium (34.9 ± 13.6 versus 41.8 ± 8.1 mEq) and potassium (3.0 ± 1.2 and 4.3 ± 1.4 mEq, for follicular and luteal phases respectively) were unaffected by menstrual phase

Blood components.

Exercise increased plasma osmolality, and the concentrations of plasma electrolytes similarly during the follicular and luteal phase (Table 11), but the increases in Hct and Hb appear slightly attenuated in the luteal phase, leading to smaller decreases in calculated percent change in plasma volume at the end of exercise (Table 11).

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APPENDIX A
Tables 1-11

	Follicular	Luteal	OC E+P	Follicular	Luteal	OC P
BW, kg	61.4 ± 4.1	61.8 ± 4.1	61.6 ± 3.8	60.7 ± 3.7	61.1 ± 3.4	60.0 ± 3.5
$P_{[E_2]}$, pg/ml (range)	27.3 ± 5.6 (12.3-40.8)	105.1 ± 26.2 (63.6-189.6)	> 12.0	26.1 ± 6.7 (13.1-36.2)	146.7 ± 38.3 (61.1-222.0)	25.1 ± 5.3 (6.4-26.7)
$P_{[P_4]}$, ng/ml (range)	1.3 ± 0.6 (0.3-2.2)	8.7 ± 3.1 (5.2-19.1)	> 0.02	0.49 ± 1.0 (0.4-0.8)	9.8 ± 2.2 (5.2-18.3)	> 0.02
P_{Osm}-$P_{[AVP]}$ slope $(pg \cdot ml^{-1}) \cdot mOsm^{-1}$	0.47 ± 0.11	0.51 ± 0.18	0.49 ± 0.12	0.49 ± 0.14	0.55 ± 0.17	0.46 ± 0.14
P_{Osm}-$P_{[AVP]}$ *x*-intercept, mOsm/kg H_2O	282 ± 1	278 ± 1*	276 ± 2#	283 ± 1	279 ± 1*	280 ± 2
P_{Osm}-thirst slope, mm/mOsm	13.7 ± 3.5	14.0 ± 2.7	13.3 ± 3.7	12.8 ± 1.7	12.9 ± 2.9	13.7 ± 2.1
P_{Osm}-thirst *x*-intercept, mm	280 ± 3	278 ± 2	276 ± 2	280 ± 1	279 ± 2	280 ± 2

Table 1. Subject characteristics.

Table 2. Blood responses at rest, during dehydration and *ad libitum* drinking.

	Pre-exercise	Exercise		Rehydration		
	0 min	150 min	0 min	60 min	120 min	180 min
$S_{[Na^+]}$, mEq/l						
Follicular	137.9 ± 0.5*#	141.3 ± 0.9*#	140.5 ± 0.9#	137.1 ± 0.5*#	136.2 ± 0.5*#	136.1 ± 0.5*#
Luteal	136.7 ± 0.6	139.6 ± 0.9	139.2 ± 0.8	136.2 ± 0.7	135.3 ± 0.5	134.9 ± 0.4
OC E + P	136.2 ± 0.6	139.9 ± 0.7	138.9 ± 0.7	135.9 ± 0.4	135.7 ± 0.4	134.8 ± 0.7
$S_{[K^+]}$, mEq/l						
Follicular	3.85 ± 0.10	4.75 ± 0.11	4.18 ± 0.06	4.31 ± 0.05	4.19 ± 0.08	4.03 ± 0.08
Luteal	3.90 ± 0.10	4.77 ± 0.08	4.18 ± 0.07	4.35 ± 0.01	4.28 ± 0.08	4.14 ± 0.08
OC E + P	4.03 ± 0.10	4.82 ± 0.10	4.31 ± 0.07	4.42 ± 0.12	4.29 ± 0.0.7	4.17 ± 0.06
TP, g/l						
Follicular	6.7 ± 0.1	7.4 ± 0.2	7.0 ± 0.1	6.6 ± 0.1	6.7 ± 0.1	6.6 ± 0.1
Luteal	6.8 ± 0.1	7.4 ± 0.2	7.0 ± 0.1	6.8 ± 0.1	6.7 ± 0.1	6.7 ± 0.1
OC E + P	6.7 ± 0.1	7.3 ± 0.2	6.9 ± 0.1	6.7 ± 0.1	6.7 ± 0.1	6.6 ± 0.2
	Pre-exercise	**Exercise**		**Rehydration**		
	0 min	150 min	0 min	60 min	120 min	180 min
$S_{[Na^+]}$, mEq/l						
Follicular	137.7 ± 0.4*§	141.3 ± 0.9*§	140.4 ± 0.6*§	137.2 ± 0.8*§	136.8 ± 0.6*§	136.3 ± 0.5*§
Luteal	136.8 ± 0.4	140.2 ± 0.9	139.1 ± 0.8	136.2 ± 0.6	135.5 ± 0.7	134.7 ± 0.5
OC P	136.6 ± 0.5	140.6 ± 1.5	139.4 ± 0.8	136.5 ± 0.7	136.5 ± 0.8	136.0 ± 0.6
$S_{[K^+]}$, mEq/l						
Follicular	3.86 ± 0.08	4.68 ± 0.11	4.14 ± 0.08	4.21 ± 0.07	4.09 ± 0.06	3.98 ± 0.04
Luteal	3.97 ± 0.08	4.94 ± 0.13	4.25 ± 0.06	4.41 ± 0.05	4.31 ± 0.07	4.02 ± 0.06
OC P	3.87 ± 0.11	4.70 ± 0.15	4.26 ± 0.13	4.12 ± 0.14	4.05 ± 0.08	3.90 ± 0.07
TP, g/l						
Follicular	6.7 ± 0.1	7.3 ± 0.2	6.8 ± 0.2	6.6 ± 0.2	6.6 ± 0.1	6.5 ± 0.1
Luteal	6.9 ± 0.1	7.5 ± 0.2	7.0 ± 0.2	6.9 ± 0.2	6.8 ± 0.2	6.8 ± 0.2
OC P	6.8 ± 0.1	7.3 ± 0.2	6.9 ± 0.1	6.7 ± 0.1	6.7 ± 0.1	6.6 ± 0.2

Table 3. Blood responses at rest, and during dehydration and *ad libitum* drinking.

	Pre-exercise	Exercise	Rehydration			
	0 min	150 min	0 min	60 min	120 min	180 min
Hct , %						
Follicular	36.3 ± 0.8$^{*\#}$	38.1 ± 0.9‡	36.6 ± 0.7	36.3 ± 0.8$^{*\#}$	36.3 ± 0.7$^{*\#}$	36.2 ± 0.8$^{*\#}$
Luteal	36.8 ± 1.0†	39.7 ± 0.8	37.7 ± 0.9†	37.1 ± 0.9†	37.0 ± 0.9†	37.0 ± 1.0†
OC E + P	35.7 ± 0.6$^{\delta}$	38.0 ± 0.8$^{\delta}$	36.4 ± 0.9$^{\delta}$	35.8 ± 0.9$^{\delta}$	35.6 ± 0.8$^{\delta}$	35.2 ± 0.7$^{\delta}$
Hb, g/dl						
Follicular	12.2 ± 0.2	13.0 ± 0.3	12.5 ± 0.3	12.1 ± 0.3	12.1 ± 0.3	12.1 ± 0.3
Luteal	12.5 ± 0.4	13.5 ± 0.4	12.8 ± 0.4	12.4 ± 0.4	12.4 ± 0.4	12.4 ± 0.4
OC E + P	11.9 ± 0.3$^{\delta}$	12.8 ± 0.3$^{\delta}$	12.1 ± 0.2$^{\delta}$	11.9 ± 0.2$^{\delta}$	11.7 ± 0.2$^{\delta}$	11.7 ± 0.2$^{\delta}$
PV, % change						
Follicular	---	-8.6 ± 1.3	-2.6 ± 1.6	1.3 ± 1.6	1.2 ± 1.7	2.5 ± 1.8
Luteal	---	-9.5 ± 2.6	-3.3 ± 2.0	0.2 ± 1.4	0.7 ± 1.6	0.5 ± 1.5
OC E + P	---	-7.9 ± 1.2	-0.5 ± 1.2	1.9 ± 1.3	3.6 ± 1.0	5.1 ± 1.7
$P_{[AVP]}$, pg/ml						
Follicular	1.3 ± 0.2	4.0 ± 0.8	3.3 ± 0.9	1.7 ± 0.4	1.6 ± 0.3	1.6 ± 0.3
Luteal	1.2 ± 0.2	3.8 ± 0.7	3.0 ± 0.7	1.5 ± 0.4	1.3 ± 0.3	1.5 ± 0.4
OC E + P	1.6 ± 0.3	3.1 ± 0.4	3.1 ± 0.4	2.7 ± 0.7	1.9 ± 0.4	2.3 ± 0.4
TP, g/l						
Follicular	6.7 ± 0.1	7.4 ± 0.2	7.0 ± 0.1	6.6 ± 0.1	6.7 ± 0.1	6.6 ± 0.1
Luteal	6.8 ± 0.1	7.4 ± 0.2	7.0 ± 0.1	6.8 ± 0.1	6.7 ± 0.1	6.7 ± 0.1
OC E + P	6.7 ± 0.1	7.3 ± 0.2	6.9 ± 0.1	6.7 ± 0.1	6.7 ± 0.1	6.6 ± 0.2

	Pre-exercise	Exercise	Rehydration			
	0 min	150 min	0 min	60 min	120 min	180 min
Hct , %						
Follicular	36.4 ± 0.7	37.5± 0.8	36.2 ± 0.7	35.8 ± 0.5	35.8 ± 0.5	35.4 ± 0.6
Luteal	37.9 ± 0.9††	40.0 ± 1.2††	38.4 ± 1.0††	37.8 ± 0.9††	37.9 ± 1.1††	37.6 ± 1.0†
OC P	37.1 ± 0.9	39.0 ± 0.9	37.2 ± 1.1	36.1 ± 0.9	36.3 ± 1.0	36.4 ± 0.8
Hb, g/dl						
Follicular	12.3 ± 0.4	13.2 ± 0.5	12.4 ± 0.4	12.1 ± 0.4	12.1 ± 0.4	11.9 ± 0.4
Luteal	13.0 ± 0.4	13.7 ± 0.5	12.9 ± 0.4	12.6 ± 0.4	12.6 ± 0.4	12.6 ± 0.4
OC P	12.6 ± 0.4	13.3 ± 0.4	12.5 ± 0.4	12.2 ± 0.3	12.2 ± 0.4	12.4 ± 0.4
PV, % change						
Follicular	---	-7.5 ± 1.2	0.0 ± 1.4	2.3 ± 1.1	3.1 ± 1.1	5.0 ± 0.7
Luteal	---	-7.4 ± 1.0	0.1 ± 1.1	3.2 ± 0.1	0.8 ± 1.2	1.6 ± 1.1
OC P	---	-6.5 ± 1.0	0.4 ± 0.9	4.7 ± 1.4	4.5 ± 1.3	5.2 ± 1.6
$P_{[AVP]}$, pg/ml						
Follicular	1.2 ± 0.4	3.7 ± 1.0	2.5 ± 0.5	1.8 ± 0.6	1.8 ± 0.6	1.6 ± 0.4
Luteal	1.1 ± 0.3	4.8 ± 1.4	2.3 ± 0.6	2.0 ± 0.5	1.9 ± 0.6	1.9 ± 0.6
OC P	1.0 ± 0.2	4.0 ± 1.2	2.7 ± 0.7	1.8 ± 0.7	2.2 ± 0.7	1.5 ± 0.4

TP, g/l						
Follicular	6.7 ± 0.1	7.3 ± 0.2	6.8 ± 0.2	6.6 ± 0.2	6.6 ± 0.1	6.5 ± 0.1
Luteal	6.9 ± 0.1	7.5 ± 0.2	7.0 ± 0.2	6.9 ± 0.2	6.8 ± 0.2	6.8 ± 0.2
OC P	6.8 ± 0.1	7.3 ± 0.2	6.9 ± 0.1	6.7 ± 0.1	6.7 ± 0.1	6.6 ± 0.2
	Pre-exercise	**Exercise**	**Rehydration**			
	0 min	150 min	0 min	60 min	120 min	180 min
Thirst, mm						
Follicular	18 ± 9	101 ± 10	100 ± 10	21 ± 8	24 ± 11	13 ± 5
Luteal	29 ± 11	100 ± 11	97 ± 12	12 ± 5	23 ± 8	7 ± 3
OC E + P	29 ± 10	94 ± 13	101 ± 12	19 ± 6	22 ± 8	17 ± 6
Thirst, mm						
Follicular	18 ± 9	101 ± 10	100 ± 10	21 ± 8	24 ± 11	13 ± 5
Luteal	29 ± 11	100 ± 11	97 ± 12	12 ± 5	23 ± 8	7 ± 3
OC P	29 ± 10	94 ± 13	101 ± 12	19 ± 6	22 ± 8	17 ± 6

Table 4. Thirst responses to dehydrating exercise.

Table 5. Renal osmoregulatory responses at rest, and during dehydration and *ad libitum* drinking.

	Pre-Exercise	End-exercise		Rehydration	
	0 min	150 min	60 min	120 min	180 min
U_V ml/min					
Follicular	3.6 ± 1.1	1.1 ± 0.2	0.7 ± 0.1	2.0 ± 0.6	2.7 ± 0.8
Luteal	4.4 ± 0.9	1.5 ± 0.2	0.5 ± 0.1	1.1 ± 0.4	1.7 ± 0.5
OC P + E	3.3 ± 0.7	0.9 ± 0.2	0.5 ± 0.0	0.6 ± 0.1	0.9 ± 0.3
U_{Osm}, mosmol/kg H_2O					
Follicular	290 ± 123	509 ± 79	790 ± 95	577 ± 135	451 ± 152
Luteal	148 ± 29	339 ± 57	833 ± 52	633 ± 125	481± 124
OC P + E	274 ± 97	502 ± 86	889 ± 48	792 ± 94	675 ± 120
U_{Osm}/P_{Osm}					
Follicular	1.0 ± 0.4	1.8 ± 0.3	3.0 ± 0.3	2.2 ± 0.5	1.8 ± 0.6
Luteal	0.5 ± 0.1	1.1 ± 0.2	3.0 ± 0.2	1.9 ± 0.5	1.9 ± 0.5
OC P + E	1.1 ± 0.4	1.7 ± 0.3	3.2 ± 0.2	2.5 ± 0.5	2.3 ± 0.5
C_{H_2O}, ml/min					
Follicular	1.7 ± 1.1	-0.5 ± 0.2	-1.0 ± 0.2	-0.4 ± 0.3	1.0 ± 0.6
Luteal	2.5 ± 0.8	0.0 ± 0.3	-1.0 ± 0.1	-0.5 ± 0.3	0.1 ± 0.5
OC P + E	1.5 ± 0.7	-0.4 ± 0.2	-1.0 ± 0.1	-1.0 ± 0.1	-0.6 ± 0.2
C_{Osm}					
Follicular	1.9 ± 0.2	1.6 ± 0.2	1.7 ± 0.2	1.7 ± 0.2	1.7 ± 0.2
Luteal	1.8 ± 0.2	1.6 ± 0.2	1.5 ± 0.2	1.6 ± 0.1	1.6 ± 0.1
OC P + E	1.7 ± 0.1	1.3 ± 0.1	1.5 ± 0.1	1.5 ± 0.2	1.4 ± 0.1
	Pre-Exercise	**End-exercise**		**Rehydration**	
	0 min	150 min	60 min	120 min	180 min
U_V ml/min					
Follicular	5.0 ± 1.2	1.3 ± 0.3	0.6 ± 0.1	1.3 ± 0.4	1.7 ± 0.5
Luteal	4.6 ± 0.8	3.5 ± 0.5	0.9 ± 0.1	1.4 ± 0.5	1.7 ± 0.5
OC P + E	4.4 ± 0.6	3.7 ± 0.7	0.8 ± 0.7	1.5 ± 0.5	2.0 ± 0.5
U_{Osm}, mosmol/kg H_2O					
Follicular	171 ± 48	410 ± 81	876 ± 77	662 ± 132	486 ± 128
Luteal	166 ± 43	406 ± 77	837 ± 55	635 ± 137	567 ± 139
OC P	125 ± 23	387 ± 58	799 ± 39	553 ± 130	415 ± 109
U_{Osm}/P_{Osm}					
Follicular	0.6 ± 0.2	1.4 ± 0.3	2.7 ± 0.5	2.6 ± 0.5	1.8 ± 0.5
Luteal	0.6 ± 0.2	1.4 ± 0.3	3.0 ± 0.2	2.2 ± 0.5	1.9 ± 0.5
OC P	0.4 ± 0.1	1.4 ± 0.2	2.8 ± 0.2	1.6 ±0.5	1.6 ± 0.4
C_{H_2O}, ml/min					
Follicular	3.0 ± 1.0	-0.1 ± 0.2	-1.1 ± 0.1	-0.5 ± 0.5	0.1 ± 0.5
Luteal	2.4 ± 0.9	-0.2 ± 0.2	-1.0 ± 0.1	-0.2 ± 0.4	0.1± 0.6
OC P	2.6 ± 0.6	-0.4 ± 0.2	-0.9 ± 0.1	0.1 ± 0.5	0.4 ± 0.5
C_{Osm}					
Follicular	2.0 ± 0.3	1.4 ± 0.3	1.6 ± 0.2	1.8 ± 0.2	1.6 ± 0.2
Luteal	2.0 ± 0.2	1.5 ± 0.1	1.5 ± 0.1	1.6 ± 0.1	1.5 ± 0.1
OC P	1.8 ± 0.2	1.6 ± 0.3	1.4 ± 0.2	1.5 ± 0.1	1.5 ± 0.3

	Pre-Exercise	End-exercise		Rehydration	
	0 min	150 min	60 min	120 min	180 min
GFR, ml/min					
Follicular	113 ± 8	83 ± 10	74 ± 10	92 ± 12	83 ± 9
Luteal	119± 5	94 ± 7	72 ± 9	84 ± 8	91 ± 11
OC E + P	111 ± 6	89 ± 13	86 ± 9	88 ± 12	91 ± 9
FE_{Na+}, %					
Follicular	0.49 ± 0.09	0.66 ± 0.16	0.93 ± 0.14	0.60 ± 0.05	0.54 ± 0.05
Luteal	0.32 ± 0.06	0.37 ± 0.09	0.65 ± 0.14	0.51 ± 0.06	0.49 ± 0.08
OC E + P	0.36 ± 0.07	0.42 ± 0.11	0.66 ± 0.10	0.62 ± 0.12	0.78 ± 0.31
U_{Na+}, mEq					
Follicular	5.2 ± 0.8	13.4 ± 2.8*#	9.3 ± 1.3	5.7 ± 0.9	4.0 ± 0.4
Luteal	3.3 ± 0.6	7.2 ± 1.5	6.0 ± 0.8	5.0 ± 1.5	4.8 ± 1.5
OC E + P	3.6 ± 0.6	7.4 ± 1.6	6.7 ± 0.8	4.3 ± 0.6	4.2 ± 0.9
U_{K+}, mEq					
Follicular	2.2 ± 0.4	10.30 ± 2.1	7.2 ± 1.4	5.2 ± 1.4	3.3 ± 0.4
Luteal	4.1 ± 1.9	11.5 ± 2.0	5.2 ± 1.2	3.4 ± 0.9	2.8 ± 0.6
OC E + P	1.9 ± 0.4	8.7 ± 1.6	5.8± 1.1	4.1± 1.1	4.1± 0.8
$[Na^+]_u/[K^+]_u$					
Follicular	2.2 ± 0.2	1.0 ± 0.2	2.5 ± 0.8	4.2 ± 2.1	1.3 ± 0.3
Luteal	1.8 ± 0.6	0.7 ± 0.2	2.4 ± 0.9	6.5 ± 4.9	1.7 ± 0.4
OC E + P	2.5 ± 0.8	0.8 ± 0.2	1.5 ± 0.3	1.2 ± 0.2	1.1 ± 0.2

	Pre-Exercise	End-exercise		Rehydration	
	0 min	150 min	60 min	120 min	180 min
GFR, ml/min					
Follicular	119 ± 9	89 ± 9	82 ± 9	85 ± 5	96 ± 4
Luteal	115 ± 8	96 ± 5	93 ± 8	111 ± 9	103 ± 9
OC P	120 ± 8	87 ± 7	79 ± 7	87 ± 6	90 ± 6
FE_{Na+}, %					
Follicular	0.36 ± 0.09	0.43 ± 0.10	0.71 ± 0.14	0.57 ± 0.11	0.46 ± 0.12
Luteal	0.35 ± 0.05	0.42 ± 0.12	0.55 ± 0.19	0.35 ± 0.07	0.33 ± 0.05
OC P	0.35 ± 0.05	0.47 ± 0.14	0.58 ± 0.10	0.44 ± 0.08	0.41 ± 0.09
U_{Na+}, mEq					
Follicular	4.5 ± 1.1	10.7 ± 2.5*§	7.6 ± 1.9	6.7 ± 1.6	4.8 ± 1.0
Luteal	4.3 ± 0.7	8.7 ± 1.8	5.9 ± 1.3	3.5 ± 0.6	3.3 ± 0.5
OC P	3.6 ± 0.8	8.5 ± 3.3	6.1 ± 1.3	3.1 ± 0.7	2.8 ± 0.6
U_{K+}, mEq					
Follicular	2.1 ± 0.7	8.0 ± 1.7	6.2 ± 1.6	4.0 ± 1.2	2.6 ± 0.4
Luteal	2.1 ± 0.5	11.2 ± 1.4	5.5 ± 0.8	3.3 ± 0.3	3.2 ± 0.5
OC P	2.0 ± 0.5	8.8 ± 2.1	3.6 ± 0.4	2.5 ± 0.5	3.0 ± 0.6
$[Na^+]_u/[K^+]_u$					
Follicular	2.9 ± 0.9	1.3 ± 0.5	2.9 ± 1.2	2.2 ± 0.6	2.1 ± 0.4
Luteal	2.6 ± 0.8	0.9 ± 0.3	1.2 ± 0.4	1.1 ± 0.3	1.0 ± 0.2
OC P	2.2 ± 0.8	0.9 ± 0.5	1.8 ± 0.7	1.7 ± 0.6	1.8 ± 0.6

Table 6. Renal electrolyte excretion at rest, during dehydration and *ad libitum* drinking.

	Exercise		Rehydration		
	Pre-	End-			
	0 min	150 min	0 min	120 min	180 min
HR, beats/min					
Follicular	77 ± 4	144 ± 6	86 ± 4	76 ± 3	75 ± 4
Luteal	75 ± 5	142 ± 5	88 ± 4	75 ± 6	80 ± 5
OC E+P	78 ± 3	135 ± 6	85 ± 6	77 ± 4	76 ± 4
MAP, mm Hg					
Follicular	83 ± 2	85 ± 3	77 ± 2	80 ± 2	79 ± 1
Luteal	82 ± 2	82 ± 3	76 ± 2	79 ± 2	78 ± 2
OC E+P	83 ± 1	84 ± 4	77 ± 2	77 ± 2	79 ± 1
SBP, mm Hg					
Follicular	113 ± 3	141 ± 7	110 ± 2	106 ± 2	108 ± 2
Luteal	115 ± 3	137 ± 6	109 ± 3	109 ± 4	109 ± 2
OC E+P	118 ± 2	145 ± 8	111 ± 2	109 ± 1	112 ± 1
DBP, mm Hg					
Follicular	69 ± 2	57 ± 2	61 ± 2	67 ± 3	64 ± 1
Luteal	66 ± 2	52 ± 3	60 ± 2	65 ± 3	62 ± 3
OC E+P	66 ± 1	54 ± 3	61 ± 2	61 ± 3	63 ± 2
PP, mm Hg					
Follicular	44 ± 3	84 ± 5	49 ± 2	39 ± 5	44 ± 2
Luteal	49 ± 5	83 ± 7	49 ± 4	44 ± 6	47 ± 4
OC E+P	52 ± 3	91 ± 6	50 ± 3	48 ± 3	49 ± 2

Table 7A. Cardiovascular responses to dehydration.

	Exercise		Rehydration		
	Pre	End			
	0 min	150 min	0 min	120 min	180 min
HR, beats/min					
Follicular	79 ± 3	145 ± 3	88 ± 4	73 ± 2	74 ± 4
Luteal	80 ± 4	142 ± 5	88 ± 4	75 ± 6	80 ± 5
OC P	81 ± 4	141 ± 7	92 ± 4	82 ± 4	81 ± 3
MAP, mm Hg					
Follicular	86 ± 2	82 ± 4	81 ± 4	78 ± 1	78 ± 2
Luteal	82 ± 2	84 ± 3	78 ± 3	79 ± 2	77 ± 2
OC P	81 ± 2	83 ± 3	78 ± 2	80 ± 2	79 ± 2
SBP, mm Hg					
Follicular	116 ± 2	140 ± 6	114 ± 4	109 ± 2	110 ± 2
Luteal	115 ± 3	137 ± 6	109 ± 3	109 ± 4	109 ± 2
OC P	116 ± 3	136 ± 4	110 ± 2	111 ± 2	110 ± 3
DBP, mm Hg					
Follicular	71 ± 3	53 ± 3	65 ± 4	62 ± 2	62 ± 3
Luteal	66 ± 2	58 ± 3	64 ± 5	63 ± 3	63 ± 2
OC P	64 ± 2	57 ± 4	62 ± 3	65 ± 2	64 ± 2
PP, mm Hg					
Follicular	45 ± 4	87 ± 5	48 ± 4	47 ± 3	49 ± 3
Luteal	42 ± 3	80 ± 5	42 ± 7	48 ± 3	43 ± 3
OC P	52 ± 4	79 ± 5	48 ± 3	47 ± 2	46 ± 3

Table 7B. Cardiovascular responses to dehydration.

	Follicular Phase		
	Pre-exercise	Exercise	Rehydration
	0 min	150 min	AUC‡
$P_{[ALD]}$, pg/ml			
Trial A	78 ± 12	275 ± 65	$228 \cdot 10^2 \pm 37 \cdot 10^2$
Trial B	96 ± 19	198 ± 47	$166 \cdot 10^2 \pm 30 \cdot 10^2$
PRA, $ng \cdot ml^{-1}$ ANG$\cdot hr^{-1}$			
Trial A	0.8 ± 0.2	3.9 ± 1.0	287 ± 60
Trial B	0.9 ± 0.2	3.4 ± 1.1	267 ± 62
$P_{[AVP]}$, pg/ml			
Trial A	1.3 ± 0.2	3.7 ± 0.8	399 ± 72
Trial B	1.2 ± 0.4	3.5 ± 0.8	374 ± 106
$P_{[ANP]}$, pg/ml			
Trial A	33.0 ± 3.9	88.1 ± 11.7	$78 \cdot 10^2 \pm 8 \cdot 10^2$
Trial B	38.0 ± 5.3	87.9 ± 12.1	$76 \cdot 10^2 \pm 8 \cdot 10^2$

	Luteal Phase		
	Pre-exercise	Exercise	Rehydration
	0 min	150 min	AUC‡
$P_{[ALD]}$, pg/ml			
Trial A	156.8 ± 21.8*	388.1 ± 43.1*	$330 \cdot 10^2 \pm 47 \cdot 10^2$*
Trial B	154.9 ± 20.6*	499.8 ± 51.0*	$460 \cdot 10^2 \pm 52 \cdot 10^2$*
PRA, $ng \cdot ml^{-1}$ ANG$\cdot hr^{-1}$			
Trial A	1.8 ± 0.4*	6.1 ± 1.7*	471 ± 113*
Trial B	1.7 ± 0.2*	4.2 ± 0.9*	653 ± 121*
$P_{[AVP]}$, pg/ml			
Trial A	1.2 ± 0.2	3.2 ± 0.6	347 ± 79
Trial B	1.1 ± 0.3	3.7 ± 1.1	496 ± 125
$P_{[ANP]}$, pg/ml			
Trial A	49.6 ± 5.6	109.2 ± 14.5	$94 \cdot 10^2 \pm 9 \cdot 10^2$
Trial B	54.6 ± 9.2	114.8 ± 22.2	$101 \cdot 10^2 \pm 14 \cdot 10^2$

Table 8. Fluid regulation hormones over two menstrual cycles.

	Cronbach's α	
	Follicular Phase	**Luteal Phase**
Resting $P_{[AVP]}$	0.49	0.25
Exercise $P_{[AVP]}$	0.81†	0.98†
Rehydration $P_{[AVP]}$	0.58	0.96†
$P_{[AVP]}$-P_{Osm} slope	0.96†	0.81†
$P_{[AVP]}$-P_{Osm} intercept	0.90†	0.86†
Resting $P_{[ANP]}$	0.80†	0.80†
Exercise $P_{[ANP]}$	0.90†	0.87†
Rehydration $P_{[ANP]}$	0.93†	0.80†
Resting PRA	0.49	0.51
Exercise PRA	0.72	0.89†
Rehydration PRA	0.67	0.95†
Resting $P_{[ALD]}$	0.55	0.66
Exercise $P_{[ALD]}$	0.66	0.82†
Rehydration $P_{[ALD]}$	0.64	0.76
Resting $P_{[E_2]}$	0.85†	0.93†
Resting $P_{[P_4]}$	0.62	0.92†

Table 9. Reliability of fluid regulation hormones over two menstrual cycles.

	End Control	End Passive Heating	Exercise	
			20 min	40 min
HR, beats/min				
Follicular	65 ± 4	69 ± 5	144 ± 13	148 ± 14
Luteal	70 ± 1	76 ± 3	143 ± 10	152 ± 12
SV, ml				
Follicular	79 ± 6	81 ± 4	100 ± 16	106 ± 20
Luteal	83 ± 3	82 ± 4	108 ± 15	108 ± 14
CO, l/min				
Follicular	5.2 ± 0.6	5.6 ± 0.5	14.1 ± 1.7	15.3 ± 2.6
Luteal	6.1 ± 0.5	6.2 ± 0.5	15.4 ± 1.5	16.1 ± 1.7
MAP, mm Hg				
Follicular	82 ± 7	79 ± 6	79 ± 5	79 ± 6
Luteal	76 ± 4	77 ± 4	87 ± 3	83 ± 7
SBP, mm Hg				
Follicular	110 ± 6	108 ± 6	129 ± 5	124 ± 8
Luteal	105 ± 5	105 ± 6	146 ± 5	144 ± 9
DBP, mm Hg				
Follicular	60 ± 9	64 ± 6	54 ± 5	57 ± 5
Luteal	62 ± 3	63 ± 4	58 ± 6	53 ± 7
PP, mm Hg				
Follicular	42 ± 6	44 ± 1	75 ± 6	67 ± 3
Luteal	43 ± 3	42 ± 6	88 ± 10	91 ± 3

Table 10. Cardiovascular responses to passive heat and exercise (n=3).

	End Control	End Passive Heating	Exercise		
			10 min	25 min	40 min
Hct, %					
Follicular	37.7 ± 1.5	37.9± 1.6	39.3 ± 1.8	39.6 ± 1.8	40.1 ± 1.6
Luteal	38.4 ± 1.2	38.4 ± 1.1	39.8 ± 0.6	39.6 ± 1.2	39.8 ± 0.9
Hb, g/l					
Follicular	13.1 ± 0.5	13.3 ± 0.7	13.8 ± 0.7	14.0 ± 0.7	14.0 ± 0.7
Luteal	13.2 ± 0.3	13.2 ± 0.3	13.8 ± 0.4	13.8 ± 0.4	14.0 ± 0.4
PV, % Δ					
Follicular	--	-1.4 ± 1.4	-7.2 ± 2.0	-9.1 ± 1.7	-10.1 ± 2.0
Luteal	--	-0.1 ± 0.2	-6.4 ± 1.4	-5.3 ± 2.3	-7.6 ± 2.6
P_{Osm}, mm Hg					
Follicular	283 ± 1	281 ± 1	286 ± 1	286 ± 0	287 ± 1
Luteal	284 ± 2	282 ± 2	288 ± 1	286 ± 2	287 ± 2
$S_{[Na+]}$, mEq/l					
Follicular	136.2 ± 0.7	134.9 ± 0.9	137.2 ± 0.4	137.0 ± 0.7	138.1 ± 0.9
Luteal	137.7 ± 0.7	136.3 ± 1.1	138.1 ± 1.1	137.9 ± 1.3	138.8 ± 1.6
$S_{[K+]}$, mEq/l					
Follicular	3.56 ± 0.06	3.88 ± 0.11	4.43 ± 0.18	4.71 ± 0.19	4.72 ± 0.22
Luteal	3.98 ± 0.06	4.15 ± 0.06	4.65 ± 0.10	4.81 ± 0.13	4.83 ± 0.12

Table 11. Blood responses to passive heat and exercise (n=3).

Text to tables

Table 1. Subject characteristics and responses to dehydration.Pre-exercise body weight (BW) and plasma concentrations of endogenous 17-β estradiol ($P_{[E_2]}$) and progesterone ($P_{[P_4]}$) in the early follicular and mid-luteal phases of the menstrual cycle and during administration of combined (estradiol + progestin, OC E+P) and (progestin only, OC P) oral contraceptive pills. Slopes and abscissal intercepts of the individual subjects' plasma arginine vasopressin concentration ($P_{[AVP]}$)-plasma osmolality (P_{Osm}) and thirst-P_{Osm} relationships during dehydration in the early follicular and mid-luteal phases of the menstrual cycle and OC E+P and OC P. *Difference between the follicular and luteal phases. #Difference between follicular phase and OC E+P. Differences were considered statistically significant at $P < 0.05$. Data are expressed as mean ± SEM.

Table 2. Blood responses at rest, and during dehydration and *ad libitum* drinking. Serum concentrations of sodium ($S_{[Na+]}$) and potassium $S_{[K+]}$), and total protein concentration (TP). *Difference between the follicular and luteal phases. #Difference between follicular phase and OC E+P, §Difference between follicular phase and OC P. Differences were accepted as significant at $P < 0.05$. Data are expressed as mean ± SEM.

Table 3. Blood responses at rest, and during dehydration and *ad libitum* drinking. Hematocrit (Hct), blood hemoglobin concentration (Hb), plasma volume (PV), plasma arginine vasopressin concentration ($P_{[AVP]}$) and total protein (TP).. *Difference between the follicular and luteal phases. #Difference between follicular phase and OC E+P, †Difference between luteal phase and OC E+P. §Difference between follicular phase and OC P. ††Difference between luteal phase and OC P. δDifference between OC E+P and OC P. Differences were accepted as significant at $P < 0.05$. Data are expressed as mean ± SEM.

Table 4. Thirst ratings (analog-rating scale) at rest, and during dehydration and *ad libitum* drinking.

Table 5. Renal osmoregulatory responses at rest, and during dehydration and *ad libitum* drinking. Urine flow (U_v), urine osmolality (U_{Osm}), plasma osmolality (P_{Osm}), free water clearance (C_{H_2O}), osmolar clearance (C_{Osm}). #Difference between follicular phase and OC E+P, †Difference between luteal phase and OC E+P. Data are expressed as mean ± SEM.

Table 6. Renal function and electrolyte excretion at rest, during dehydration and *ad libitum* drinking. Glomerular filtration rate (GFR), fractional excretion of sodium (FE_{Na+}), urine excretion of sodium (U_{Na+}) and potassium (U_{K+}), and ratio of urine sodium and potassium concentrations ($[Na^+]_u/[K^+]_u$). *Difference between the follicular and luteal phases. #Difference between follicular phase and OC E+P. §Difference between follicular phase and OC P. Differences were accepted as significant at $P < 0.05$. Data are expressed as mean ± SEM.

Table 7A. Cardiovascular responses to dehydration. Heart rate (HR), mean (MAP), systolic (SBP), diastolic (DBP) and pulse (PP) blood pressures at rest and in response to 150 min dehydrating exercise and 180 of *ad libitum* rehydration in the follicular and luteal phases, and

during combined estradiol/progestin oral contraception administration OC E+P, n=8). Data are expressed as mean ± SEM.

Table 7B. Cardiovascular responses to dehydration. Heart rate (HR), mean (MAP), systolic (SBP), diastolic (DBP) and pulse (PP) blood pressures at rest and in response to 150 min dehydrating exercise and 180 of *ad libitum* rehydration in the follicular and luteal phases, and during progestin-only oral contraception administration (n=9). Data are expressed as mean ± SEM.

Table 8. Fluid regulation hormones over two menstrual cycles. Trial A and Trial B are the first and second trials within the specified menstrual phase. Plasma renin activity (PRA) and plasma concentrations of aldosterone ($P_{[ALD]}$), arginine vasopressin ($P_{[AVP]}$) and atrial natriuretic peptide ($P_{[ANP]}$) in response to dehydrating exercise and 180 min of *ad libitum* rehydration in the early follicular and mid-luteal phases of the menstrual cycle. *Difference between the follicular and luteal phases. ‡Area under the curve (AUC, trapezoid). Differences were accepted as significant at $P < 0.05$. Data are expressed as mean ± SEM.

Table 9. Reliability of fluid regulation hormones over two menstrual cycles. Cronbach's α for reliability within two follicular and two luteal phase tests. †Cronbach's $\alpha \geq 0.80$ was considered reliable.

Table 10. Cardiovascular responses to passive heat and exercise. Heart rate (HR), stroke volume (SV), cardiac output (CO) mean (MAP), systolic (SBP), diastolic (DBP) and pulse (PP) blood pressures at rest (27°C) and in response to 20 min of passive heating (35°C) and 40 min of exercise (35°C) in the follicular and luteal menstrual phases (n=3). Data are expressed as mean ± SEM.

Table 11. Blood responses to passive heat and exercise. Hematocrit (Hct), blood hemoglobin concentration ([Hb]), percent change plasma volume from 27°C control (ΔPV), plasma osmolality (P_{Osm}), and serum concentrations of sodium ($S_{[Na+]}$) and potassium ($S_{[K+]}$) at rest (27°C) and in response to 20 min of passive heating (35°C) and 40 min of exercise (35°C) in the follicular and luteal menstrual phases (n=3). Data are expressed as mean ± SEM.

Text to figures

Figure 1. Time line for sex hormone administration. (Figure imbedded in text).

Figure 2. Plasma osmolality (P_{Osm}) at rest, and in response to dehydrating exercise and 180 min of *ad libitum* rehydration in the follicular and luteal phases, and during combined estradiol/progestin (OC E+P, n=8) and progestin-only oral contraception administration (OC P, n=9). *Difference between the follicular and luteal phases. #Difference between follicular phase and OC E+P. §Difference between follicular phase and OC P. Differences were accepted as significant at $P < 0.05$. Data are expressed as mean ± SEM.

Figure 3. Plasma renin activity (PRA) at rest, and in response to dehydrating exercise and 180 min of *ad libitum* rehydration in the follicular and luteal phases, and during combined estradiol/progestin (OC E+P n=8) and progestin-only oral contraception administration (OC P, n=9). *Difference between the follicular and luteal phases. #Difference between follicular phase and OC E+P. †Difference between luteal phase and OC E+P. Differences were accepted as significant at $P < 0.05$. Data are expressed as mean ± SEM.

Figure 4. Plasma aldosterone concentration ($P_{[ALD]}$) at rest, and in response to dehydrating exercise and 180 min of *ad libitum* rehydration in the follicular and luteal phases, and during combined estradiol/progestin (OC E+P n=8) and progestin-only oral contraception administration (OC P, n=9). *Difference between the follicular and luteal phases. †Difference between luteal phase and OC E+P. ††Difference between luteal phase and OC P. Differences were accepted as significant at $P < 0.05$. Data are expressed as mean ± SEM.

Figure 5. Plasma atrial natriuretic peptide ($P_{[ANP]}$) at rest, and in response to dehydrating exercise and 180 min of *ad libitum* rehydration in the follicular and luteal phases, and during combined estradiol/progestin (OC E+P n=8) and progestin-only oral contraception administration (OC P, n=9). *Difference between the follicular and luteal phases. †Difference between luteal phase and OC E+P. §Difference between follicular phase and OC P. Differences were accepted as significant at $P < 0.05$. Data are expressed as mean ± SEM.

Figure 6. Mean plasma arginine vasopressin concentration ($P_{[AVP]}$) responses to increases in plasma osmolality (P_{Osm}) during dehydration in the follicular and luteal phases, and during combined estradiol/progestin (OC E+P, n=8) and progestin-only oral contraception administration (OC P, n=9). Data are expressed as mean ± SEM.

Figure 7. Cumulative renal sodium (Na^+) in response to dehydrating exercise and 180 min of *ad libitum* rehydration in the follicular and luteal phases, and during combined estradiol/progestin (OC E+P n=8) and progestin-only oral contraception administration (OC P, n=9). *Difference between the follicular and luteal phases. #Difference between follicular phase and OC E+P. §Difference between follicular phase and OC P. Differences were accepted as significant at $P < 0.05$. Data are expressed as mean ± SEM.

Figure 8. Body fluid balance after dehydrating exercise and during 180 min of *ad libitum* rehydration in the follicular and luteal phases, and during combined estradiol/progestin (OC E+P, n=8) and progestin-only oral contraception administration (OC P, n=9). #Difference between the follicular and OC E+P. †Difference between luteal phase and OC E+P. Differences were accepted as significant at $P < 0.05$. Data are expressed as mean ± SEM.

Figure 9. Esophageal (T_{es}) and weighted skin temperature (T_{sk}) during control (27°C), passive heating (35°C) and exercise in the heat (35°C). (Figure embedded in text).

Figure 10. Arm sweat rate during control (27°C), passive heating (35°C) and exercise in the heat (35°C). (Figure embedded in text).

Figure 11. Arm sweat rate as a function of temperature changes during exercise at 35°C. (Figure embedded in text).

APPENDIX B
Figures 2-8

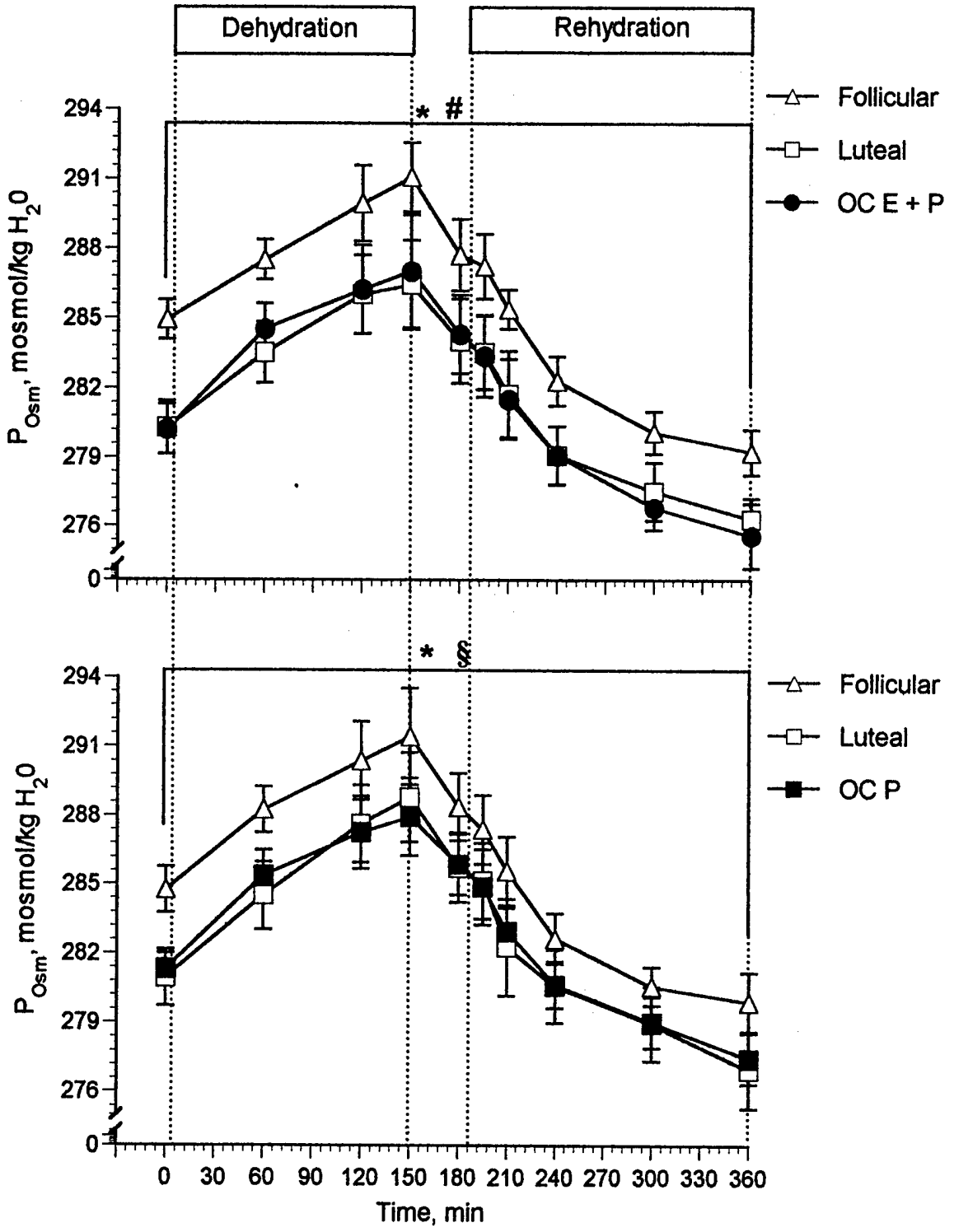

Figure 2. Plasma osmolality at rest, dehydration and rehydration

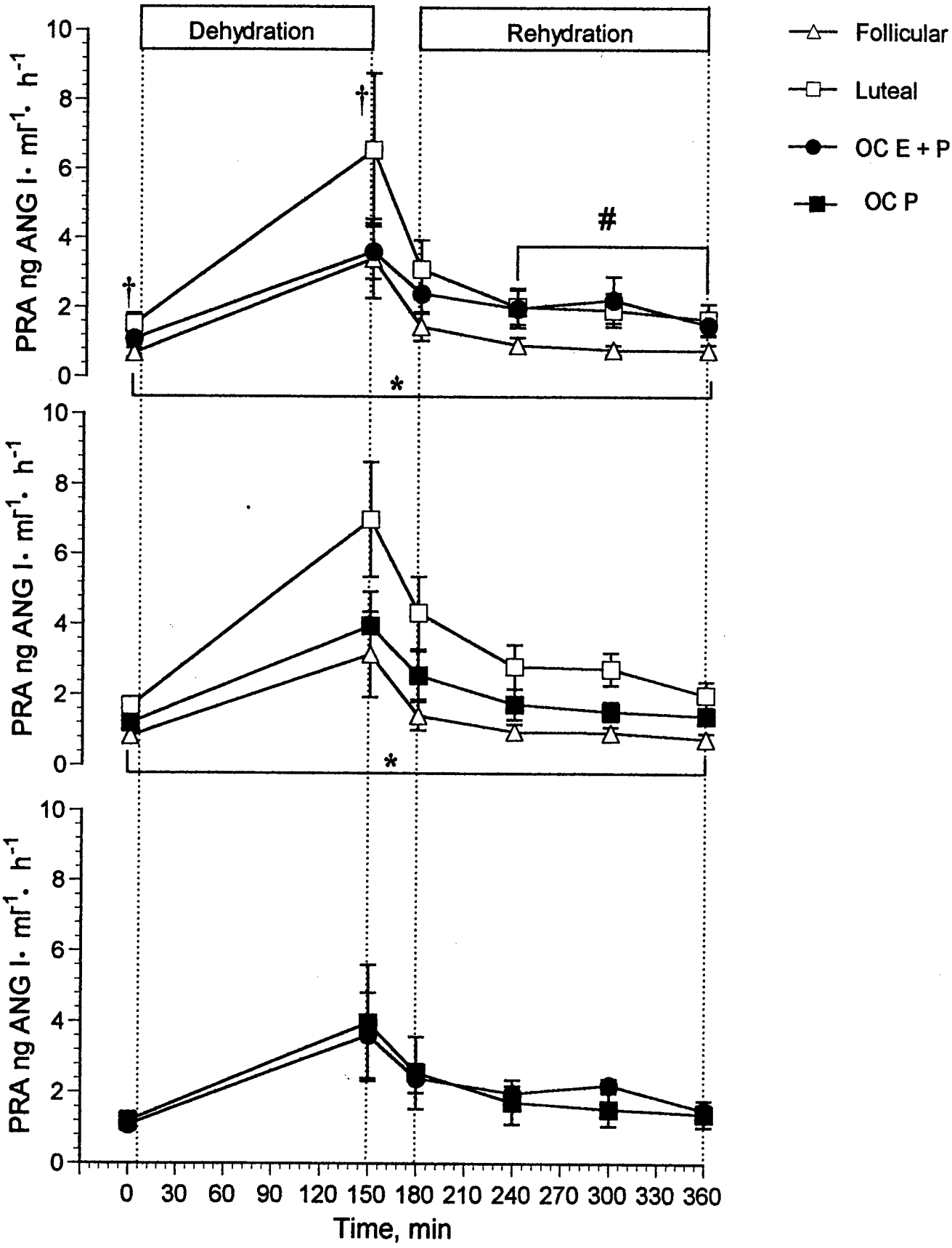

Figure 3. Plasma renin activity at rest, dehydration and rehydration

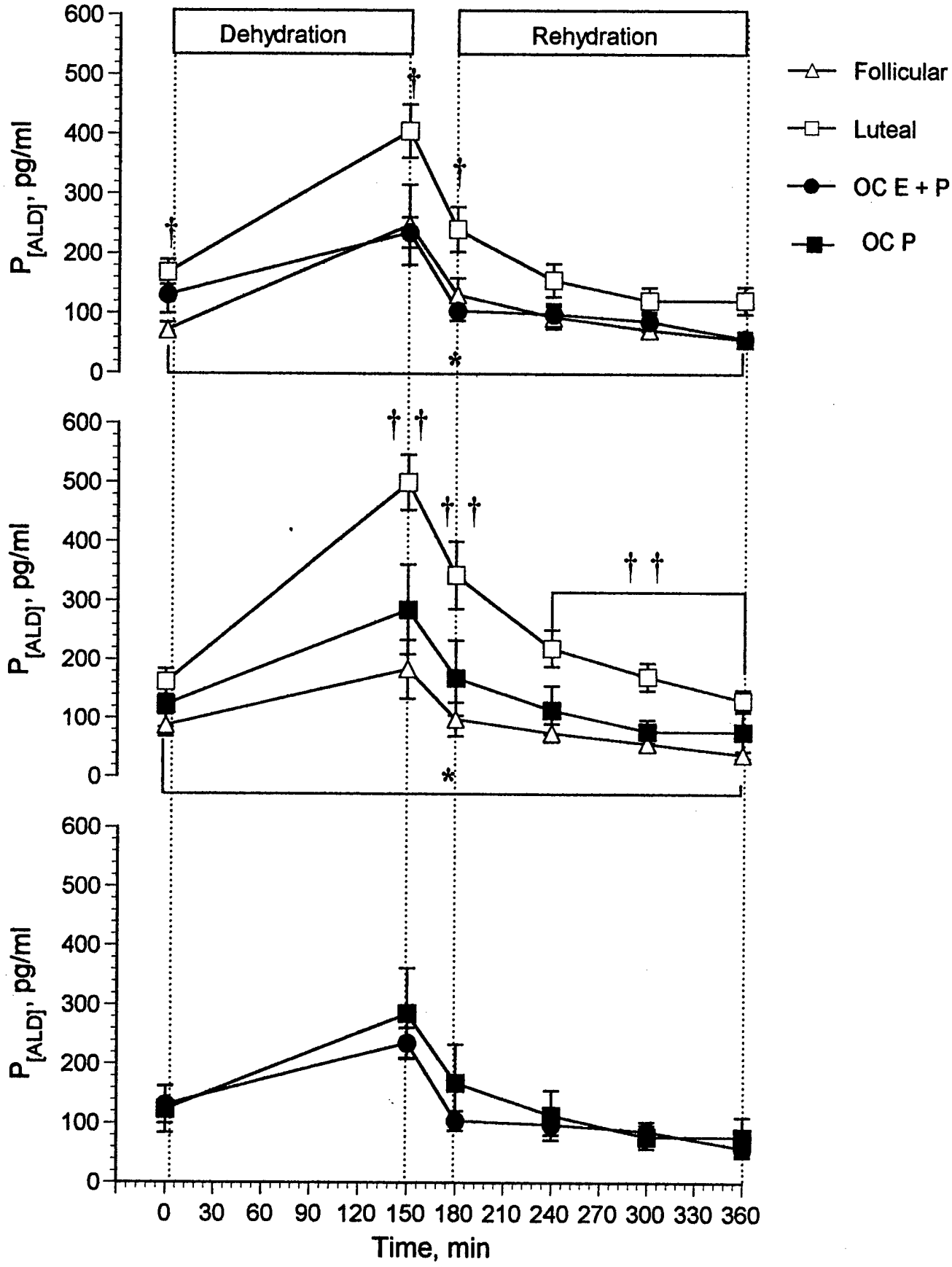

Figure 4. Plasma aldosterone concentration at rest, dehydration and rehydration

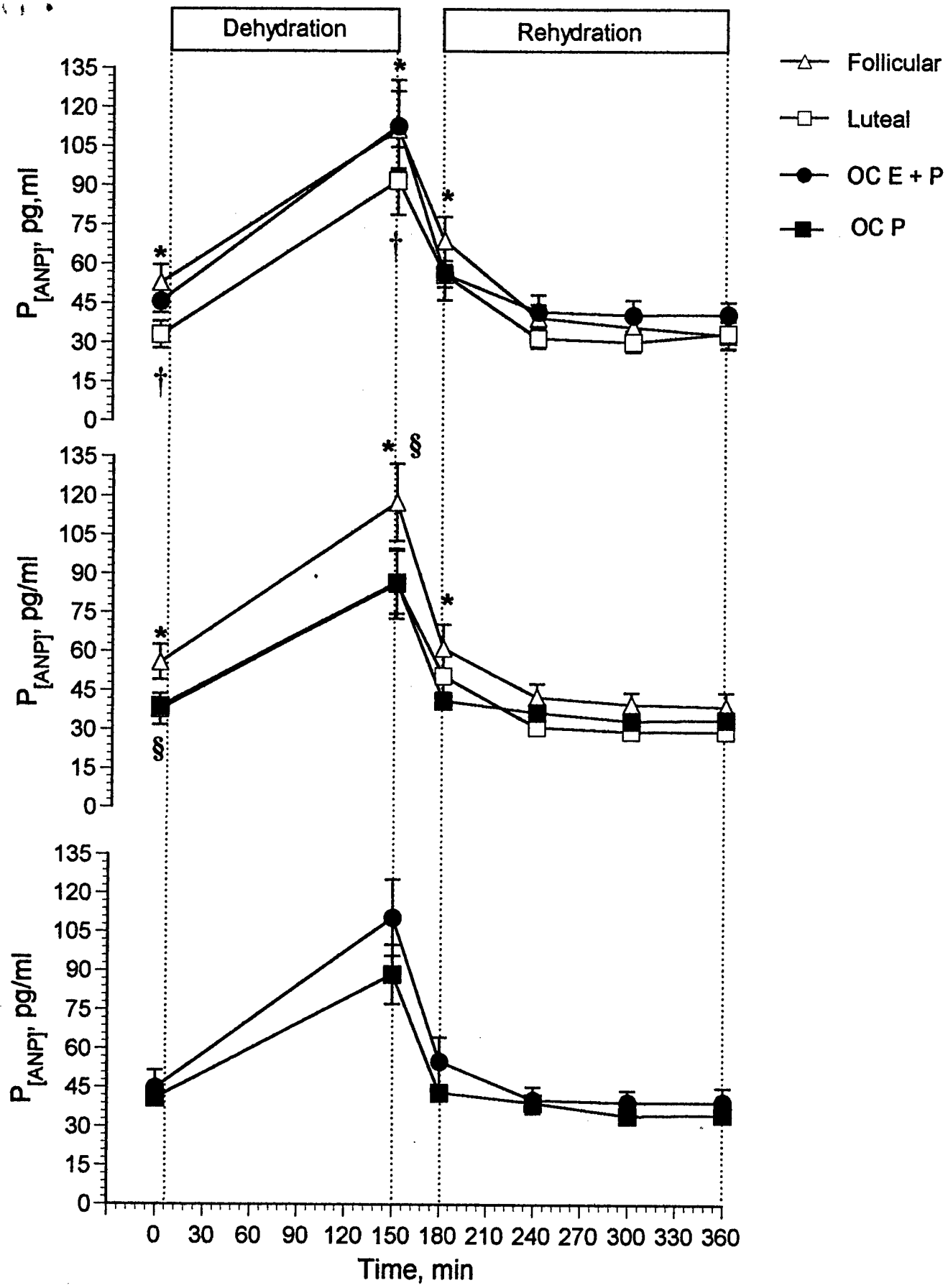

Figure 5. Plasma atrial natriuratic peptide concentration at rest, dehydration and rehydration

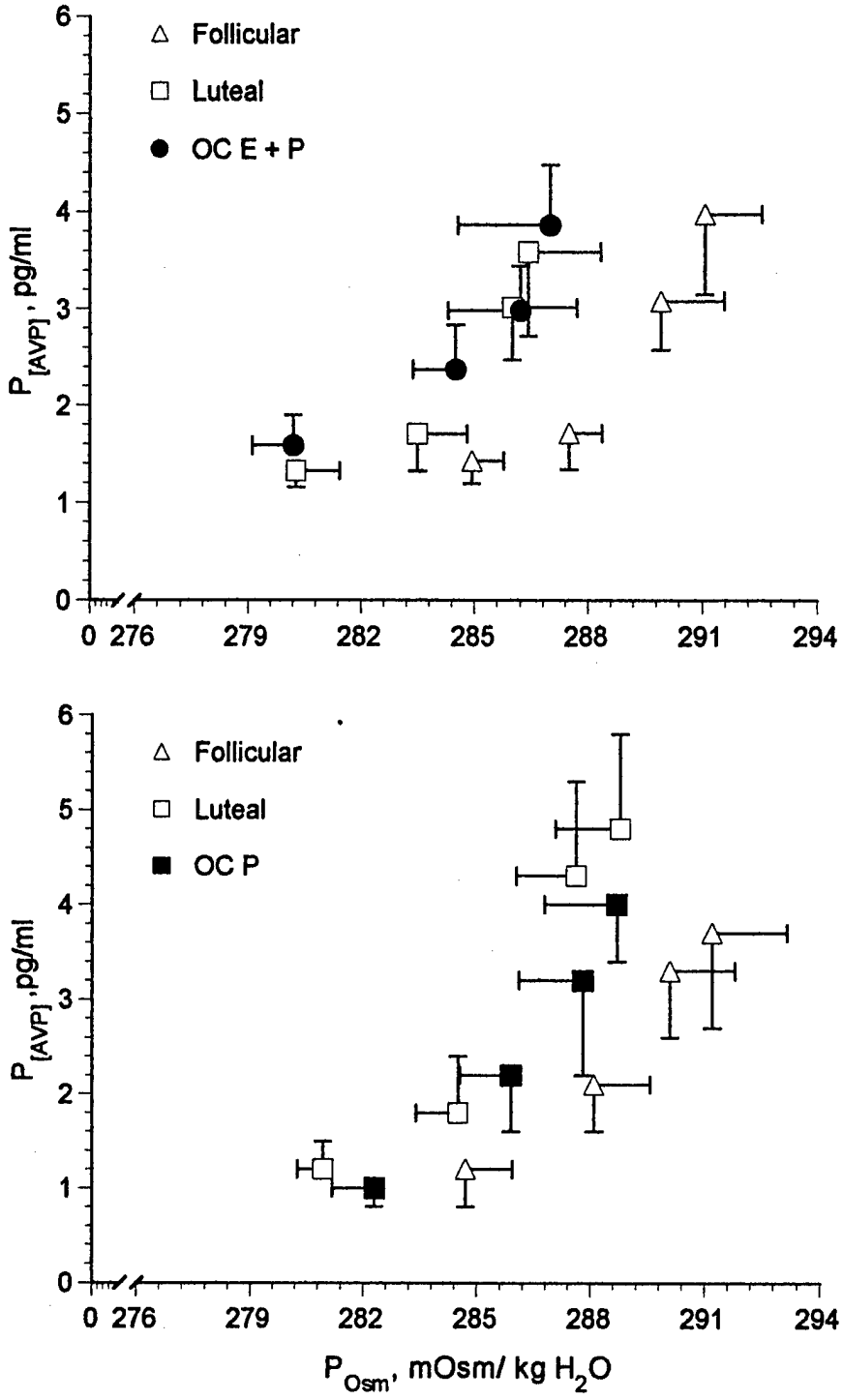

Figure 6. Osmotic regulation of arginine vasopressin during dehdyration.

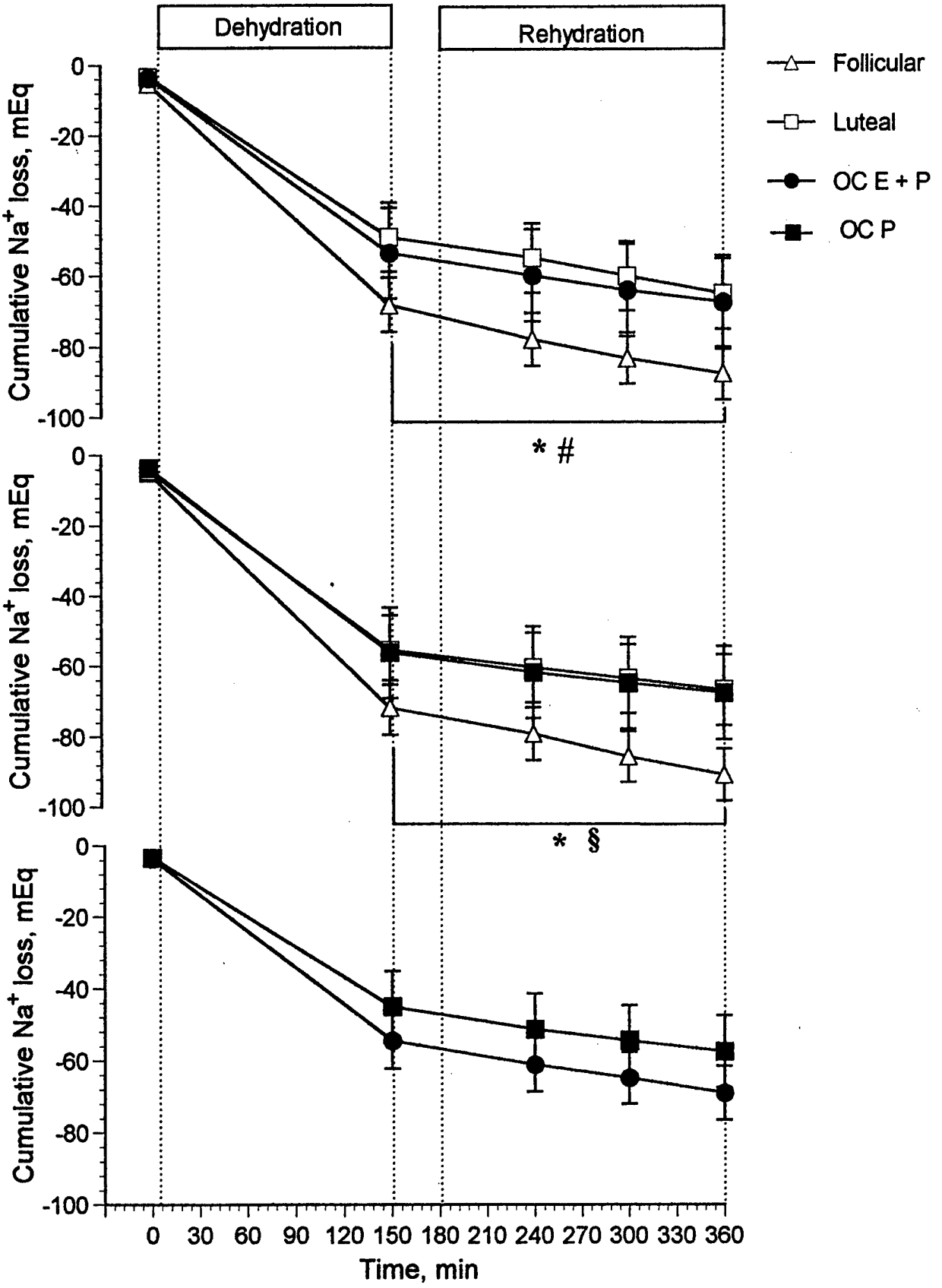

Figure 7. Cumulative sodium loss

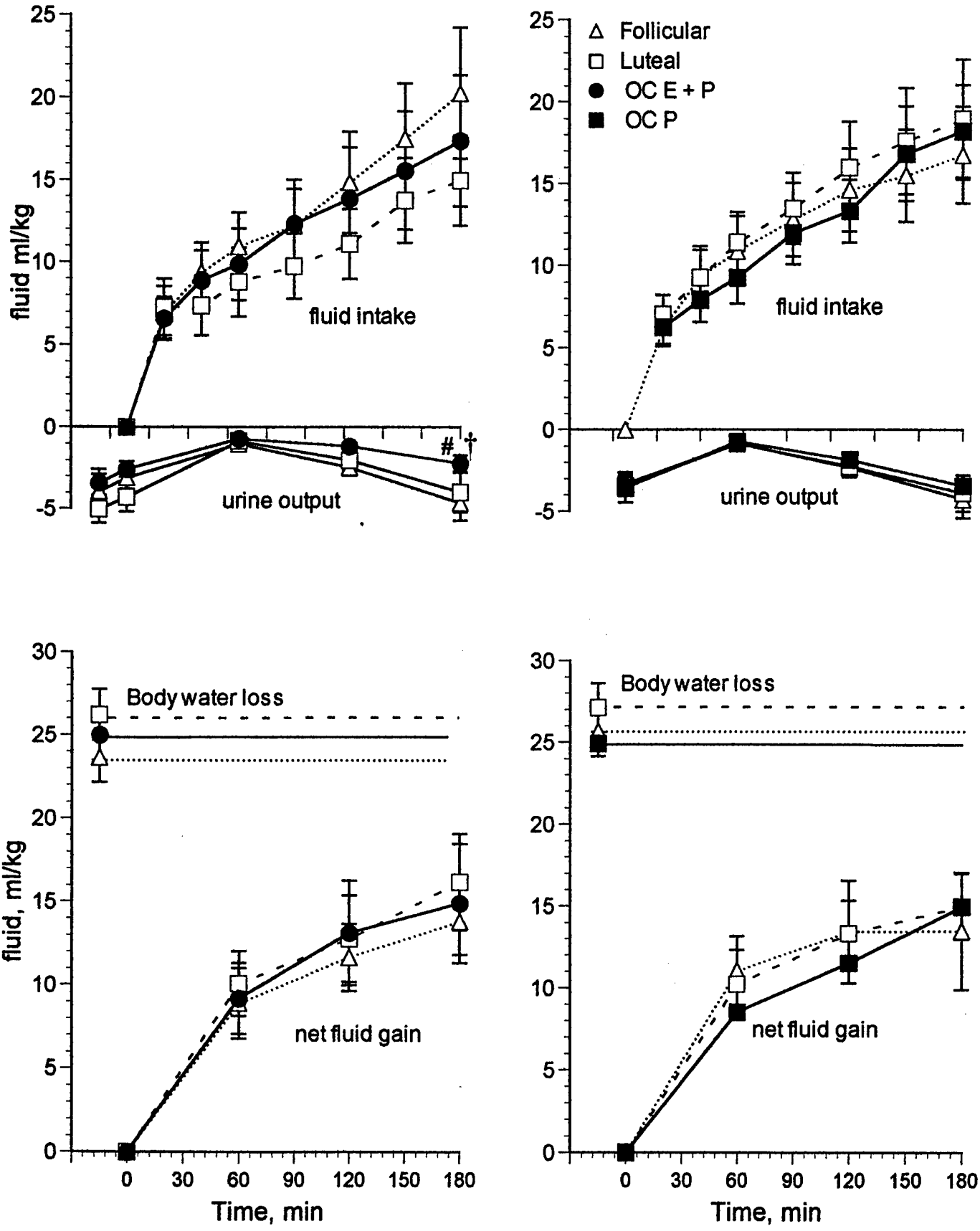

Figure 8. Body fluid balance